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BIOLOGICAL MONITORING OF CHEMICAL CONTAMINATION AT CRAB ORCHARD NATIONAL WILDLIFE REFUGE

by

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FINAL REPORT

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ABSTRACT

Several hazardous waste sites have been identified at Crab Orchard National Wildlife Refuge near Carterville, IL. Area 9 Landfill, Job Corp Landfill, and the Old Refuge Shop have soil contaminated with polychlorinated biphenyls, lead and cadmium. The objectives of this research were to establish baseline levels for these contaminants in terrestrial biota, determine the extent of biological effects, and establish the degree of ecological risk posed by the sites.

PCBs, but not lead, readily move from contaminated soils into the terrestrial community. Pre-remediation levels were established in this report for June beetle adults, tree bark, honeysuckle, white-footed mice, starlings and Canada geese. June beetles and tree bark are particularly good monitoring organisms because they are relatively easy to collect and will probably be only moderately impacted by remediation activities.

PCBs at the landfills were associated with biological effects in earthworms, white-footed mice, and starlings. Acute risk was apparent for soil dwelling invertebrates. Hepatic effects were evident in white-footed mice occupying Area 9 Landfill and Job Corp Landfill. Carcass concentrations of PCBs found in white-footed mice were also likely to be associated with decreased reproduction, based on laboratory studies. Reproductive effects, in the form of reduced hatching success, were documented in starlings occupying nest boxes next to Area 9 Landfill. The reduced hatch appeared to be related to decreased parental attentativeness rather than direct effects on embryos.

The ecological risk assessment clearly establishes risk to individual organisms associated with landfills at Crab Orchard NWR, especially those containing PCBs. Individual risk is high for organisms occurring on or near the landfills. The risk of population decline over a large geographic area is

lower than individual risk, since the landfills comprise a relatively small portion of the surface area. Consequently, localized effects will likely be compensated for when considering larger spatial scales.

These data provide background information on contaminant levels in biota at Crab Orchard National Wildlife Refuge and, in some cases, information on the types of effects that wildlife are experiencing as a result of the chemical contamination. Post-remediation studies will be able to use these data to provide evidence as to the success or effectiveness of the cleanup activities.

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INTRODUCTION

Crab Orchard National Wildlife Refuge (NWR) was created in 1946 and occupies 17,420 hectares in southern Illinois. Prior to 1946 the area was used as farmland and, during World War II, as an industrial area for the manufacture of munitions. Subsequent to 1946 other industries have utilized the area for manufacturing munitions, metal fabrication, plating, and the manufacture of printing inks and electrical components. Wastes from these industries were accumulated in several landfills on the Refuge.

A statewide survey of metal contamination in livers from hunter harvested white-tailed deer indicated significant elevation of nickel and lead concentrations in animals collected at Crab Orchard NWR (Woolf *et al.*, 1983). Additionally, routine monitoring of fish from Crab Orchard Lake identified elevated levels of mercury as early as 1977 (Hite and King, 1977). Subsequent investigations focused on identifying the sources of these contaminants at Crab Orchard NWR.

Preliminary investigations of the landfills at Crab Orchard NWR indicated sufficient chemical contamination to warrant inclusion, in 1984, of several sites on the National Priorities List established by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)(USEPA, 1988). A Remedial Investigation of these landfills was initiated by U.S. Fish and Wildlife Service and Sangamo Weston, Inc. and has recently been completed and released (O'Brien and Gere, 1988).

The Remedial Investigation was an extensive analysis of the type, magnitude, and distribution of contamination at 19 sites in Crab Orchard NWR. Seven sites containing various amounts of polychlorinated biphenyls (PCBs), lead, cadmium, chromium, mercury, magnesium, and cyanide were selected for remediation: Area 9 Landfill, Area 9 Building Complex, Job Corps Landfill,

Old Refuge Shop, Fire Station Landfill, Water Tower Landfill, and Area 7 Plating Pond. Human health hazards were judged to be low principally because most sites are closed to the public. Five of the seven sites, however, were identified to pose moderate or high risks to wildlife and the contaminant characteristics of these sites are shown in Table 1. The location of Area 9 Landfill, Job Corp Landfill, Old Refuge Shop, and reference sites are shown in Figure 1.

O'Brien and Gere (1988) established endangerment to wildlife at certain sites at Crab Orchard NWR using a risk assessment approach. The assessment of ecological risk can be separated into three components: exposure assessment, effects assessment, and risk estimation (USEPA, 1989a; 1989b). Wildlife exposure to PCBs can be assessed by modelling uptake from environmental mediums, measuring body burdens of chemicals in the field (Moriarty, 1988), or measuring specific biomarkers which reflect exposure to a particular chemical (McCarthy and Shugart, 1990). Biological effects can be assessed by measuring levels of chemicals that produce changes in health and performance of individuals, populations, or communities. Frequently used measures of biological effects include survival, growth, reproduction, population structure, community structure, and biomarkers of effects (Suter, 1993). Estimation of risk is calculated by comparing exposure and biological effects data.

Two aspects of the ecological risk assessment performed by O'Brien and Gere (1988) need refinement in order to better define the extent of wildlife endangerment at Crab Orchard NWR. First, calculation of risk was based on exposure levels for wildlife predicted by modelling uptake of chemicals from soil (O'Brien and Gere, 1988, Exhibit D). No attempts were made to verify these levels of exposure in the field. Secondly, biological effects data used in risk calculations were based on biological effects in laboratory animals and may have limited relevance in natural systems. The objectives of this

research parallel these two aspects of the O'Brien and Gere risk assessment and attempt to refine the data used to calculate risk to wildlife.

The Feasibility Study and subsequent Record of Decision require remediation of the PCB Operable Units by incineration, Metals Operable Units by stabilization and fixation, and co-contaminated sites by a combination of these methodologies. The goal of this research project was twofold. First, information is needed regarding current levels of contamination in wildlife at Crab Orchard NWR, especially near Area 9 Landfill, Job Corp Landfill, and Old Refuge Shop. This information can be used to judge the effectiveness of remediation with regard to wildlife contamination. Second, information is needed regarding the degree of biological effects in wildlife inhabiting contaminated areas at the refuge. Specific objectives of the proposed research are outlined below:

1. to establish a database for levels of chemical contamination in certain environmental receptors at the Area 9 Landfill (including the building complex), Job Corp Landfill, and Old Refuge Shop.

2. to determine the extent and magnitude of biological effects in wildlife associated with hazardous waste chemicals at Crab Orchard NWR.

3. to summarize ecological risk associated with chemical contamination at Crab Orchard NWR.

Table 1. Contaminant characteristics of hazardous waste sites identified as posing moderate to high risk for wildlife[a].

Site identification	Type of Contaminant	Environmental Medium	Maximum Contamination (mg/kg soil dry wt.)
Area 9 Landfill	PCBs	Surface soil	13,000 (wet wt.)
	lead	Surface soil	8,270
	mercury	Surface soil	35 ppb
	chromium	Surface soil	< Bkgd
Area 9 Buildings	PCBs	Surface soil	120,000 (wet wt.)
Job Corp Landfill	PCBs	Surface soil	69,042
	lead	Surface soil	17,410
	cadmium	Surface soil	57
Old Refuge Shop	cadmium	Stream sediments	780
	cyanide	Stream sediments	181
Fire Station Landfill	lead	Surface soil	2,355
	magnesium	Surface soil	40,268
	mercury	Surface soil	290 ppb

[a] Data from O'Brien and Gere (1988)

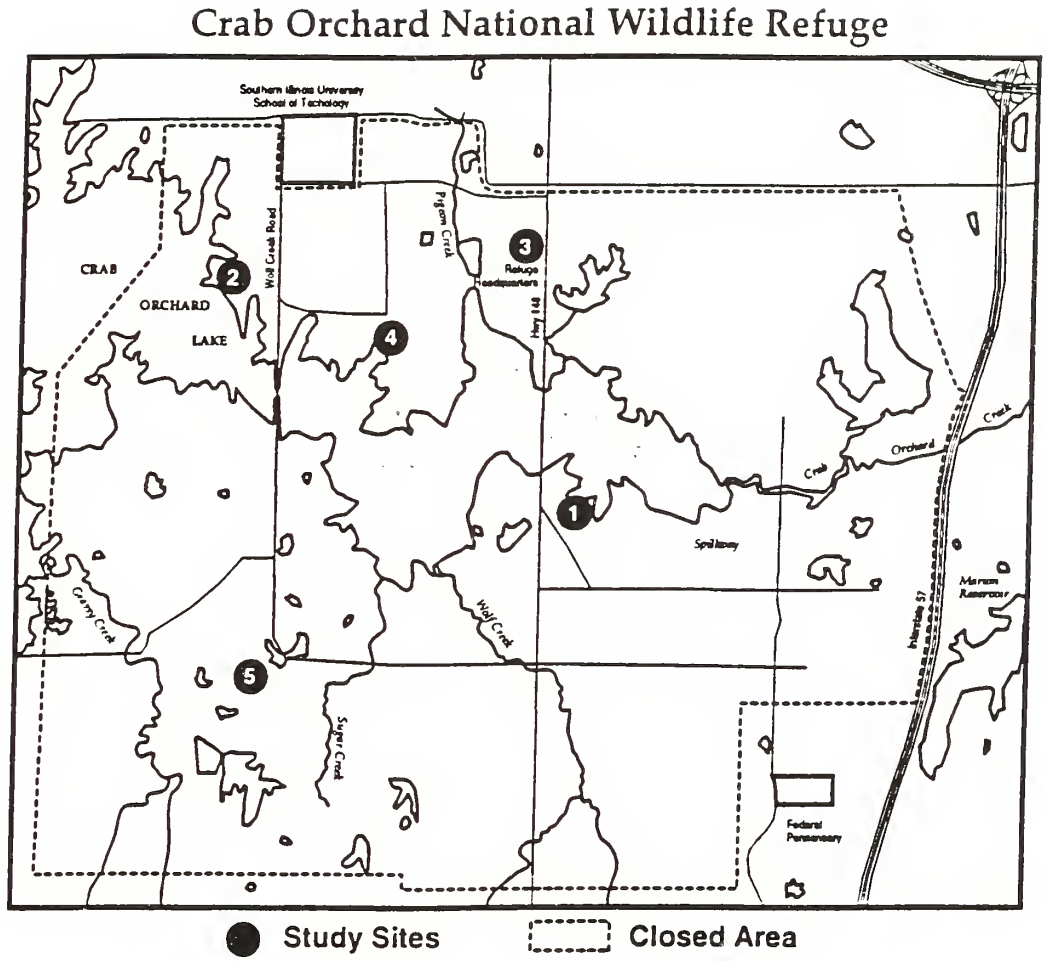

Figure 1. Location of study sites at Crab Orchard NWR. 1) Area 9 Landfill; 2) Job Corp Landfill; 3) Old Refuge Shop; 4) Olin reference site; and 5) Area 13 reference site.

DESCRIPTION OF SITES (Maps of sites are in Appendix I)

Crab Orchard NWR is located at the southern terminus of the Illinoisan glaciation (Frye, 1965) which resulted in considerable topographic variation in the region. In general, areas north of the refuge were glaciated producing flat relief suitable for farming. Areas south of the refuge tend to be hilly with rock outcroppings, much of which is unsuitable for agriculture. Several manmade lakes are present on the refuge. Crab Orchard Lake, constructed in 1940, is the largest lake accounting for about 16% (2,800 hectares) of surface area at the refuge.

Area 9 Landfill

Area 9 is a manufacturing site on the refuge. It was leased to Sangamo Electric Co., Capacitor Division, from 1946 to 1962 and is currently leased to Olin Corporation. Area 9 comprises an inactive landfill (Area 9 Landfill) (Map I, Appendix I) and the Area 9 Building Complex directly west of the landfill. Crab Orchard Lake is about 100 m northeast of the landfill and runoff can enter the lake through several intermittent creeks. Sangamo Electric manufactured various types of capacitors which utilized aluminum, electrolytes, mica, silver, lead foil, and PCBs. Olin Corporation currently uses the buildings to manufacture explosives.

The landfill was closed in 1964 after about two decades as a repository for industrial waste (O'Brien and Gere, 1988). The limits of the landfill are discernible by changes in the topography and vegetation, revealing an area of approximately 1 hectare (2.5 acres) with an estimated fill thickness of 8 to 10 feet in the middle and 6 feet at the edges (O'Brien and Gere, 1988). The volume of the landfill is estimated to be from 16,000 to 35,000 cubic yards. About 80% of the landfill has vegetation cover dominated by plants in the genera *Phragmites* (reed grasses) and *Solidago* (goldenrod). Materials

visible on the surface appear to be electrical components consisting of small capacitors, capacitor parts, chunks of golden resin, and a number of 3-inch steel cuplike pieces. A magnetometer survey suggests that the majority of wastes are buried along the eastern and northern edges of the landfill (Map 1, Appendix I).

The Area 9 Building Complex is adjacent to the west border of Area 9 Landfill. The buildings were occupied by Sangamo Electric Co. between 1946 and 1962 and are currently occupied by Olin Corporation. Soil samples collected adjacent to several buildings in the complex contained PCB levels above 50 mg/kg wet weight. Some isolated samples collected along the sides of the access road to the Area 9 Landfill also contained elevated PCB concentrations (O'Brien and Gere, 1988).

Job Corp Landfill

The Job Corp Landfill (JCL), about 0.5 hectares in size, was active from 1951 to 1960 (O'Brien and Gere, 1988). The landfill is located adjacent to a 3 hectare lake constructed in the 1940s by the federal Job Corp (Map 2, Appendix I). The landfill is almost completely covered with a variety of herbaceous and woody plants, however, widespread debris, such as bottles, cans, mica flakes, small electrical contacts, and a few small capacitors, can be observed on the surface of the ground.

The landfill was discovered during investigation of a Canada goose dieoff in 1985. About 30 geese carcasses in various stages of decomposition were found floating on the water or littering the shores. The Fish and Wildlife Service has completed chemical analyses of these carcasses and has not identified a potential causative agent. Conclusive evidence for the etiological agent in the dieoff was never obtained (O'Brien and Gere, 1988).

Old Refuge Shop

The Old Refuge Shop (ORS) site is located adjacent to the Old Refuge Headquarters (Map 3, Appendix I). Pine wood poles were treated in a fenced area of the shop with pentachlorophenol wood preservative and shipped to various locations throughout the county, according to the refuge manager (O'Brien and Gere, 1988). Contaminants were identified in a small drainage pool located immediately north of ORS, which drains through the woods to the northwest and, ultimately, to Crab Orchard Lake.

Control Sites

Two control sites were established for this investigation. The Olin Site (OLS)(Figure 1 and Map 4 in Appendix I) was selected because it is similar in proximity, vegetation cover, and topography to Area 9 Landfill. In some cases, we needed to collect animals that were not abundant at OLS. In these cases we utilized a control site on the southwest part of the refuge (Figure 1) in an area designated as Area 13.

METHODOLOGY

Summary of samples collected-

This report contains residue information for PCBs, lead, and cadmium in a variety of environmental receptors including soil, plants, honey bees, June beetles, white-footed mice, starlings, and Canada geese. These biomonitors were collected at Area 9 Landfill and Building Complex (A9L), Job Corps Landfill (JCL), Old Refuge Shop (ORS), and reference sites shown in Maps 1-4 (Appendix I), respectively. Two control sites were utilized: the Olin Site (OLS) on the north side of the lake (Figure 1 and Map 4 in Appendix I); and Area 13 (A13) which is southwest of the lake (Figure 1). Total number of tissue samples collected and reported in this report is shown in Table 2.

Table 2. Summary of samples collected for PCB, lead, and cadmium residue analysis at Crab Orchard NWR.

	Number of Samples[a]				Total samples
Type of sample	A9L	JCL	ORS	OLS/A13	per type
Soil	15	0	0	3	18
Tree bark	9	0	0	0	9
Honeysuckle	5	0	0	3	8
Honey bees	11	0	11	11	33
June beetles	52	2	2	2	58
White-footed mice	28	17	0	26	71
Starlings	31	0	0	39	70
Canada geese	8	8	0	0	32 (2 tissue types)
TOTAL					**299**

[a] PCB analysis was performed on all samples. Lead and cadmium analyses were performed on some, but not all samples. Individuals were analyzed for mice, starlings, and geese. Most others were composites.

Collection of soil (Area 9 Landfill only)

Fifteen soil samples were collected along two transects established on Area 9 Landfill (Map 1, Appendix I). Eight soil samples were collected on each transect at 5, 25, 75, and 125 m away from the intersection (except on the west transect which did not have the 125 m sampling location). To prevent "hot spot" bias, soil samples comprised 3 subsamples collected at the apices of a 1 m equilateral triangle for each sampling location. The soil surface was cleared of debris and 3 subsamples were taken at each sampling location using a hammer sampler fitted with a 2 X 2 inch removable stainless steel sleeve.

Soil collected in the metal sleeve from each of the three subsamples was added to a pre-cleaned stainless steel mixing bowl and mixed for about 1 min to increase homogeneity in the composite sample. Soil was then transferred to a pre-cleaned and pre-labeled glass container. Soil samples were stored on ice until returned to the laboratory. Three samples were also collected from the reference area (Olin site) for background and quality control analyses. Three samples from Area 9 Landfill were split during the collection process so that soil samples could be sent to different analytical laboratories for quality assurance. Split samples were analyzed at the Animal Disease Laboratory, Centralia, IL and Patuxent Wildlife Research Center, Patuxent, MD.

Collection of plants (Area 9 Landfill only)

Bark samples- The bark of pin oak, *Quercus palustris* was sampled as a biomonitor of aerial deposition of PCBs. Three trees, greater than 10 inches in diameter at breast height, were sampled at each of three locations: 1) The Landfill site- the three pin oak trees located closest to the point that the east-west transect intersects the eastern edge of the landfill were

sampled; 2) Upwind site- three trees located about 0.5 km west of Area 9 Landfill were sampled; and 3) Downwind site- three trees located about 0.5 km east of Area 9 Landfill were sampled. Each tree sample comprised six subsamples collected equidistant around the circumference of the tree at breast height. A 25 cm^2 subsample of bark was removed using a pre-cleaned stainless steel 5 cm wood chisel. The six subsamples were combined into one sample which was analyzed for PCBs and lead.

Honeysuckle- Five foliage samples of honeysuckle, *Lonicera japonica*, (25 g per plant per sample) were collected from Area 9 Landfill. One sample was collected near the intersection of the transects shown in Map 1, Appendix I. The remaining 4 samples were collected on each transect as it radiates out from the landfill. Exact locations of plants collected were recorded and mapped. Three samples were collected at the Olin reference site. Samples were collected by stripping foliage from the stem and placing in prelabeled containers. Latex gloves were worn during the sample collection and gloves were decontaminated between samples.

Collection of honey bees (Area 9 Landfill, Old Refuge Shop, and Control)

Hives of the honey bee, *Apis mellifera*, were established in May 1990 at Area 9 Landfill, the Old Refuge Shop, and the Olin reference site. Three samples (30 g each) of brood bees from each of these hives were collected in June 1991. Bees were collected by removing frames from the brood chamber and shaking bees into a funnel leading to a collection box. Bees were transported to the laboratory in a ventilated (screened) box, sacrificed by CO_2, weighed into three 30 g replicates, and stored in glass containers until chemical analysis. Bees were analyzed for cadmium, lead, and PCBs.

In September of 1991, foragers were sampled. Forager bees were collected by placing a screen across the entrance to the hive and vacuuming

bees from the screen (Hoover wet/dry cleaner- series 300) as they returned from foraging trips. Trapped bees were placed in collection box and returned to the laboratory and processed as described above. Three samples containing 10 g each were collected from the Old Refuge Shop, Area 9 Landfill, and the Olin reference site. Forager bees were again sampled from August to September of 1992. Five samples containing 10 g each were collected from the Old Refuge Shop, Area 9 Landfill, and the Olin reference site.

Collection of beetles (Area 9 Landfill, Old Refuge Shop, Reference)

The initial collection of June (or May) beetles was made during the period from April through June 1991. Attempts were made to collect three 30 g samples at Area 9 Landfill, the Old Refuge Shop, and the Olin reference site. UV portable light traps (Carolina Biological Supply) were used to collect adult beetles. The 10 V light traps were actuated about 0.5 hrs after dusk by tripping a connector to a battery pack using mechanical timers. About 500 ml of deionized and distilled water was added to each trap. Beetles entering the trap were wetted by the water and unable to fly. Traps were placed near the landfills (within 10 m). Beetles were collected from the traps in early morning and returned to the laboratory, weighed, sacrificed using CO_2, keyed to species, and frozen for subsequent chemical analysis. Species identification was principally based on aedeagus morphology according to Luginbill and Painter (1953) and Dillon and Dillon (1961).

June beetles were again sampled from April through June 1992, however, a different experimental design was used. Area 9 Landfill was the only site sampled in 1992 and traps were set at three different distances from the landfill; 0 m, 50 m, and 500 m. This experimental design allowed determination of the extent that June beetles were contaminated at various distances from the landfill.

Collection of white-footed mice (Area 9 Landfill, Job Corp Landfill, and reference)

White-footed mice, *Peromyscus leucopus*, were collected at Area 9 Landfill, Job Corp Landfill, and a reference site (Area 13). Transects were established for each of the sites (see Map 1 and Map 2 in Appendix I for the locations of transects at Area 9 Landfill and Job Corp Landfill, respectively). Forty-nine sample locations were established for each of the four transects extending from the point of intersection. The format for locating traps on each transect was as follows: 25 traps were located at 10 m intervals from the intersection of the transects to 250 m; on both sides of this line of traps, parallel rows of traps were set from 30 m to 80 m at 10 m intervals and at 200 m to 250 m at 10 m intervals. This design gave a linear transect of traps emanating from the intersection and provided two 3 by 6 grids at 30 m and 200 m from the intersection. One of the four transects was shorter than the other three because of obstructions at each site: this short transect had only 20 traps. Each of the transects were trapped for three nights and one transect per site was trapped on a particular night to prevent confounding results by trapping sites at different times. Trapped animals were noted as to the particular location captured and returned to the laboratory. Animals were weighed, sexed, aged, and sacrificed by cervical dislocation. Livers were quickly removed, weighed, frozen in liquid nitrogen, and stored at -70°C. Prior to freezing, a 5 mm wedge of the liver was removed and preserved in a 10% buffered formalin solution. General necropsy information was recorded along with kidney, spleen and adrenal weights. The carcasses were stored at -20°C until analyzed for PCBs.

Collection of Canada geese (Area 9 Landfill and Job Corp Landfill)

Sixteen Canada geese, *Branta canadensis*, were collected by refuge personnel in January 1991: eight from the embayment near Area 9 Landfill; and

eight near the pond at the Job Corp Landfill. Geese were taken to the laboratory, plucked, and necropsied. Samples of breast muscle (with skin) and subcutaneous fat were retained for lead and PCB analysis. A total of 32 samples were analyzed.

Collection of starlings (Area 9 Landfill and reference)

European starlings, *Sternus vulgaris*, were used as a model for fate and effects of contaminants at Area 9 Landfill. Starlings are useful monitors at hazardous waste sites because they reproduce in nest boxes affording some control over distribution of *in situ* populations. Nestlings reared in these nest boxes are fed a variety of invertebrates collected by adults in the nesting area. Biological effects information can be attained by looking at reproductive success of the birds through monitoring egg production and number of fledglings.

In 1991, a total of 18 nest boxes were placed at the Area 9 Landfill (Map 1, Appendix I) and 9 were placed at a Olin reference site (Map 4, Appendix I). Each nest box was observed twice weekly until egg laying began, then nests were monitored daily to determine time to completion of clutch, the precise hatch date, and the number hatched. Nestlings were marked using toe clips and individual weights were measured on Day 3, Day 6, Day 9, Day 12, and Day 15. Weights were collected by placing the nestlings in a nylon stocking and suspending on a spring tension weighing scale. Nestlings were collected just prior to fledging, placed in a collection box, transported to the laboratory, and sacrificed using thoracic pressure. Measurements in the laboratory were collected on wing length, primary sheath length, length of fifth primary feather, and right tarsus length. Livers were removed, weighed, sampled for histopathology, and frozen at -70°C. Liver enzyme activities are being performed at Patuxent Wildlife Center and are not included in this report. Remaining carcasses were retained for residue analysis.

Eggs that did not hatch were collected and returned to the laboratory. The eggs were weighed and length and width were determined using a micrometer. The eggs were fractured and the contents weighed and inspected to determine the approximate age of the embryo.

Collection of quail

At initiation of this research project we intended to release and recapture bobwhite quail at Area 9 Landfill. Several preliminary investigations were attempted to determine the potential success of using this methodology. In the first experiment, we released three pinioned birds fitted with radiotransmitters on collars at the Area 13 reference site and Area 9 Landfill. These birds were depredated within three days of release. We were able to recover the radios and the majority of tissue for birds killed at Area 9 Landfill. Birds killed at Area 13 were completely consumed, but we were able to recover the radios. The second experiment involved the release of five non-pinioned birds with radiocollars at Area 13 and Area 9 Landfill. Three of the five were alive on day three after release at both sites, but by day five post-release, all birds were dead. We were able to recover 9 of the 10 radios, but no birds were recovered. No results data will be presented for bobwhite quail.

Biological effects on earthworms

Earthworms, *Eisenia foetida*, were used to determine the toxicity of soil collected *in situ* according to standard methods developed for hazardous waste site assessments (USEPA, 1989c). Four soil samples from Area 9 Landfill were evaluated for soil toxicity to earthworms. Toxicity was assessed using soil samples collected at the 25 m location of each of the transects as shown in Map 1 in Appendix 1.

The assay was conducted in triplicate and included the four hazardous waste soil samples, a negative control, and a positive control containing 300 ppm of Aroclor 1254. Soil from the Olin reference site was used as the negative control. Each test replicate contained 70% artificial soil mixed with 30% of either hazardous waste soil for test samples, reference site soil for the negative control, or PCB spiked reference site soil for the positive control. The artificial soil consisted of 10% finely ground sphagnum peat, 20% kaolinite clay, 69% fine silica sand, and 1% $CaCO_3$ with a final pH between 5.5 and 6.0. The mixture of sample soil and artificial soil was hydrated to a final moisture content of 20% by weight.

The bioassay consisted of 18 glass test containers distributed evenly across the six treatment levels. Eight adult earthworms weighing between 300 and 550 mg were selected and randomly added to each container. Test containers were held in an environmental chamber at 20° ± 2°C and 50% ± 5% relative humidity. Temperature, relative humidity, and weight of the entire test container were recorded daily. The total weight of the container was used as an indicator of water loss by the container. Survival and group worm weight were recorded for each replicate on Days 0, 7, and 14.

Biological effects on white-footed mice

PCBs are strong inducers of monooxygenase enzymes (Chen and Dubois, 1973; Litterest *et al.*, 1972; Turner and Green, 1974; Villanueve *et al.*, 1971) affording this enzyme system utility as a monitor of exposure and potential environmental effects. Hepatic monooxygenase activity was measured as an indicator of biological effects. Livers collected as described above were removed from the freezer, homogenized, and the microsomal fractions obtained by ultracentrifugation as described by Mazel (1971). Conversion of ethoxy- and pentoxyresorufin was used as described by Lubet *et al.* (1985) and modified by Simmons and McKee (1992) for white-footed mice. Briefly, reactions were

performed in disposable acrylic cuvettes with 2 ml of pH 7.4 Tris-HCl buffer containing 0.025 M $MgCl_2$, 100 µg microsomal protein, 125 µM NADPH, and initiated with 10.0 µM of substrate at 37°C. Each liver was analyzed in triplicate and quantified by fluorescence at 530 nm excitation/585 nm emission on a Hitachi F-2000 fluorescence spectrophotometer.

Biological effects on starlings

Nestlings surviving to near fledgling size (15 days post-hatch) were collected, transported to the laboratory in a pre-labeled collection box, and sacrificed by thoracic pressure. Livers were immediately removed, weighed, frozen in liquid nitrogen, and stored at -70°C. Prior to freezing, a 5 mm wedge was removed and preserved in a 10% buffered formalin solution. General necropsy information was recorded along with kidney, spleen and adrenal weights. Monooxygenase activity will be assayed at Patuxent Wildlife Center, Patuxent, MD.

Reproductive success of starlings was monitored in the nest boxes at Area 9 Landfill and the reference area (Olin). Reproductive parameters included clutch size, % hatch, % fledge, and length of incubation period. Individual weights were measured for developing nestlings and correlation between weight gain and PCB concentration in nestlings was investigated. Morphometric measurements include sex, right tarsal length, flatwing chord, 5th primary length, and 5th primary sheath.

Chemical analysis

Soil, plants, and invertebrates were analyzed for chemical contaminants at the Animal Disease Laboratory, Illinois Department of Agriculture, Centralia, IL. Quality assurance cross-checks were performed at the Cooperative Wildlife Research Laboratory and U.S. Fish and Wildlife Service's

Patuxent Wildlife Research Center. For samples analyzed at the Animal Disease Laboratory, PCBs in tissues were extracted by homogenizing in hexane and separated from interfering compounds using gel permeation chromatography. Final preparations of PCB extracts were analyzed on a Hewlett-Packard 5890 Gas Chromatograph fitted with a capillary column and an electron-capture detector. PCBs in tissue were quantified based on content of Aroclor 1254, which is the dominant component of the soil at Crab Orchard NWR. Quantification was accomplished by comparing 10 representative peaks of a standard Aroclor 1254 preparation to the same 10 peaks in extracted samples containing unknown quantities of PCBs. Final concentrations were expressed in µg Aroclor 1254/g wet weight of tissue. Lipid content was determined gravimetrically by extracting tissue with petroleum ether, reducing to dryness, and weighing remaining lipid.

White-footed mice and starlings collected in 1990 and 1991, respectively, were analyzed at SIU's Wildlife Analytical Laboratory. For these samples, analysis of PCBs in tissues followed the method of Tanabe *et al.* (1987) as modified by Hong and Bush (1990). Samples were homogenized with anhydrous sodium sulfate at a ratio of 6:1, Na_2SO_4 to tissue. The mixture was blended and then extracted with hexane using a Soxtec continuous extractor. Five ml of concentrated H_2SO_4 was added to the hexane extract and left overnight at room temperature. The hexane fraction was collected, extracted twice with 10 ml distilled water, and reduced in volume to 1 or 2 ml.

A Hewlett-Packard 5890 II gas chromatograph equipped with a Ni-63 electron capture detector (ECD), and a 60-m Supelco SPB-5 fused silica capillary column were used to analyze the extract. A thermal gradient from 60°C to 250°C was used for elution of congeners. Nitrogen (25 psi) was used as the carrier gas and helium was used for make-up gas at a flow rate of 40 ml/min. The temperature of the injection port and electron-capture detector will be 250°C.

Duplicate samples were run through the entire analytical procedure to provide a measure of precision. Spike recoveries in uncontaminated sample material were used to assess the accuracy of the methods and to allow adjustments for recoveries, if necessary. External analytical standards (Phase Separations, Inc.) were used to quantify chemicals in samples. Periodic injection of a standard solution (every third analytical sample) was used to assure that analytical performance was acceptable. Raw data is presented in Appendix III and all records have been dated and initialed by a reviewer.

Metals analysis - Duplicate aliquots were weighed from the sample homogenate and analyzed for lead and, in samples from Old Refuge Shop, cadmium. Dried samples were acid digested in nitric acid in a teflon container. Samples were diluted in de-ionized water and analyzed on a Perkin-Elmer 4000 Atomic Absorption Spectrophotometer with HGA-graphite furnace. Quality control procedures included standard injection every fifth run and duplicate sample processing.

Moisture and lipid analysis - Moisture for each homogenate was determined gravimetrically after drying samples at 100°C for 16 hours. Lipid was measured for each homogenate by determining specific gravity using a Foss-Let Fat Analyzer or gravimetrically. Samples containing small amounts of lipid were analyzed gravimetrically using soxhlet extractions.

Data analysis

Statistical comparisons for residues, biochemistry, and other biological data will be accomplished with one-way ANOVA and the Least Significant Difference Test using Statistical Analysis System (SAS) (SAS Institute 1985a; 1985b). All statistical comparisons will be at a significance level of 0.05 and Bartlett's test will be used to test for deviations from normality prior to parametric analysis.

For white-footed mice and starlings, a two-way analysis of variance was initially used to investigate differences in several variables including body weights and tissue weights. The main effect means in the two-way ANOVA were sex and site. Initial results using the two-way ANOVA showed sex related differences in lipid content (% of body weight) for white-footed mice, so it was decided to keep sexes separate through remaining analyses. No sexual differences were observed in starling nestlings, so these data were pooled. Subsequent analysis was performed using one-way ANOVA to determine differences between sites for each sex.

Correlation analysis was used to investigate associations between body weight, relative organ/tissue weights, monooxygenase activity (mice only), and total or congener specific PCB levels for both starlings and white-footed mice. For mice, regression equations were calculated to describe the relationship between monooxygenase activity or relative liver weight and PCB concentration. Initially, linear trends were investigated using General Linear Models on SAS. These equations were tested for lack of fit (Sokal and Rohlf, 1969). Since some lack of fit was detected, curvilinearity was investigated using the polynomial $y=a+bx+cx^2$ and the SAS Nonlinear Procedure.

RESULTS

Soil

Concentrations of PCBs (as Aroclor 1254) in soils at Area 9 Landfill ranged from below detectable limits to 11,360 µg/g wet weight (Figure 2). Lead in soils at Area 9 Landfill ranged from 9.1 to 7,290 µg/g dry weight. Soil contaminant levels dropped off rapidly beyond the boundaries of the landfill, however, residues were found in soil collected off the landfill. For example, 3.6 µg PCBs/g soil was found in samples taken from the bottomland woods east of the landfill (Figure 2). Soil concentrations at the other waste sites were not investigated in this study.

The quality of analytical data was checked by comparing results from split soil samples analyzed at two different laboratories: the Animal Disease Laboratory in Centralia, IL (Principal Laboratory); and Mississippi State Chemical Laboratory (MSCL) in Mississippi, MS. High accuracy was observed at high concentrations of Aroclor 1254 in the soil (Table 3). Accuracy was lower for samples containing low levels of PCBs (i.e. sample 4-4). Aroclor 1248 and Aroclor 1254 were analyzed at MSCL showing that Aroclor 1254 was the major PCB mixture present at Area 9 Landfill; accounting for about 80% of total PCBs (Table 3). The similarity of soil PCBs to Aroclor 1254 is further demonstrated by visual comparison of a typical soil extract chromatogram to a chromatogram of the Aroclor 1254 standard (Figure 3).

Dibenzofuran and dibenzodioxin homologs were analyzed in soil sample 4-1 by MSCL. The 2,3,7,8-tetrachlorodibenzodioxin isotere was not detected, however, the 2,3,7,8-tetrachlorodibenzofuran was quantified (Table 4). The octachlorodibenzodioxin (OCDD) congener was the most abundant congener of the dibenzofurans and dibenzodioxins at 19 µg/g.

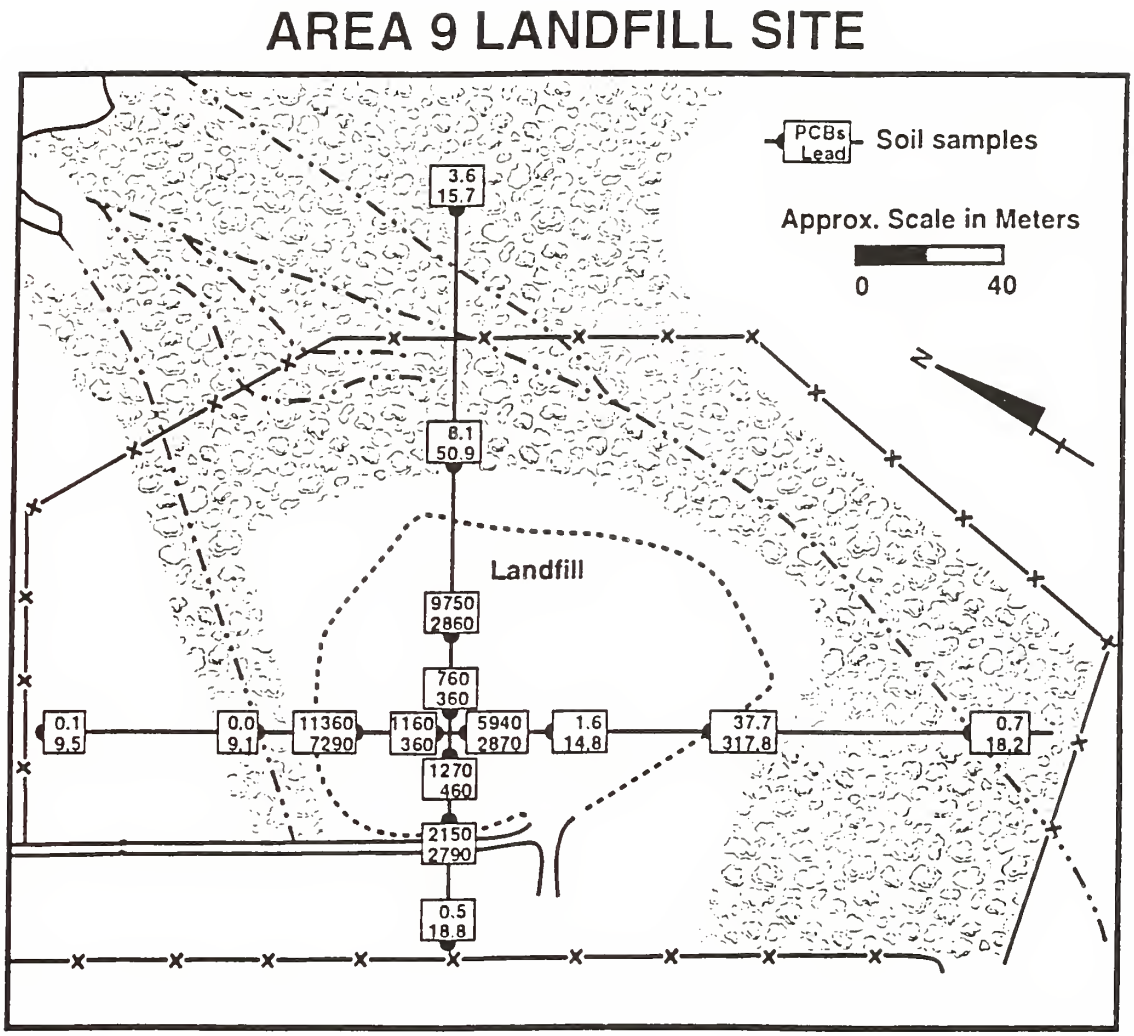

Figure 2. Soil concentration of PCBs as Aroclor 1254 (µg/g wet weight) and lead (µg/g dry weight) at Area 9 Landfill at Crab Orchard NWR.

Table 3. Comparison of chemical concentrations in soil samples from Area 9 Landfill that were split and analyzed at two different analytical laboratories.

Chemical	Soil Sample #	µg chemical/g soil (wet weight)[a] Illinois Laboratory[b]	Mississippi Laboratory[b]
Aroclor 1254	3-1	1,270	1,400
	4-1	1,160	1,300
	4-4	0.35	0.10
Aroclor 1248	3-1	NM	290
	4-1	NM	210
	4-4	NM	ND

[a] ND = not detectable; NM = not measured.

[b] Illinois laboratory was the Animal Disease Laboratory, Centralia, IL. The Mississippi laboratory was the Mississippi State Chemical Laboratory, Mississippi State, MS.

Table 4. Dibenzodioxin and dibenzofuran congener profile in soil samples collected from Area 9 Landfill at Crab Orchard NWR.

Compound	Concentration[a] µg/g wet weight
2,3,7,8-TCDD	ND
2,3,7,8-TCDF	12.97
1,2,3,7,8-PeCDD	ND
1,2,3,7,8-PeCDF	3.18
2,3,4,7,8-PeCDF	4.41
1,2,3,4,7,8-HxCDD	ND
1,2,3,6,7,8-HxCDD	ND
1,2,3,7,8,9-HxCDD	ND
1,2,3,4,7,8-HxCDF	10.48
1,2,3,6,7,8-HxCDF	4.38
2,3,4,6,7,8-HxCDF	ND
1,2,3,7,8,9-HxCDF	ND
1,2,3,4,6,7,8-HpCDD	ND
1,2,3,4,6,7,8-HpCDF	5.54
1,2,3,4,7,8,9-HpCDF	1.84
OCDD	19.15
OCDF	6.41

[a] ND = not detected. Limit of detection was 1.3 pg/g for 2,3,7,8-TCDD.

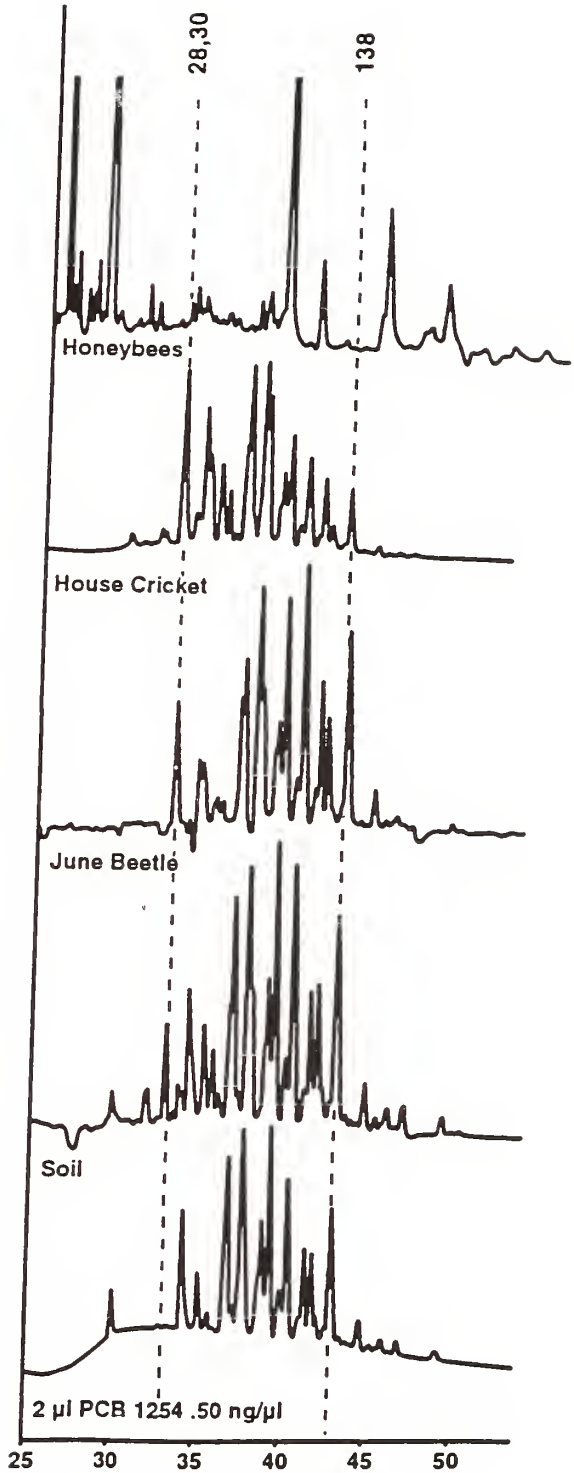

Figure 3. Gas chromatograms of standard Aroclor 1254 and extracts from soil, June beetles, house crickets (see McKee, 1992), and honey bees collected at Area 9 Landfill in Crab Orchard NWR.

Plants

Aroclor 1254 was highest in pin oak tree bark located next to the Area 9 Landfill (Figure 4). Trees sampled upwind and downwind from the landfill did not have detectable levels of PCBs (detectable limit was 0.01 µg/g). Means and standard deviations (n=3) for lead levels in tree bark were 1.89 (0.59), 1.36 (0.61), and 1.97 (0.74) µg/g dry weight for upwind, landfill and downwind locations, which demonstrates no strong tendency for lead to move from the landfill. Foliage stripped from the main stem of honeysuckle at Area 9 Landfill contained both Aroclor 1254 and lead, although no clear trends relating location and residue levels was obvious (Figure 4). No attempts were made to distinguish between surface deposition versus internalized contaminants. Plant tissue was also collected at the Olin reference site. PCB levels at the reference site were below detectable limits. The mean and standard deviation (n=3) for lead in honeysuckle foliage from the reference site was 0.38 (0.41) µg/g dry weight. Tree bark was not analyzed at the reference site.

Beetles

In 1991, light traps were used to collect beetles at the Area 9 Landfill, the Job Corp Landfill, the Old Refuge Shop and the Olin reference site. Only one species of June beetle, *Phyllophaga fervida*, was collected in sufficient numbers in 1991 to allow chemical analysis. Aroclor 1254 was identified at the low ppm range in beetles collected at Area 9 Landfill and Job Corp Landfill (Table 5). The Old Refuge Shop and reference site (Olin) were below detectable levels. Beetles from the reference site and the Old Refuge Shop were analyzed for cadmium. Despite contamination in the Old Refuge Shop soils, cadmium was not observed to bioaccumulate in beetles collected by light traps in the general vicinity.

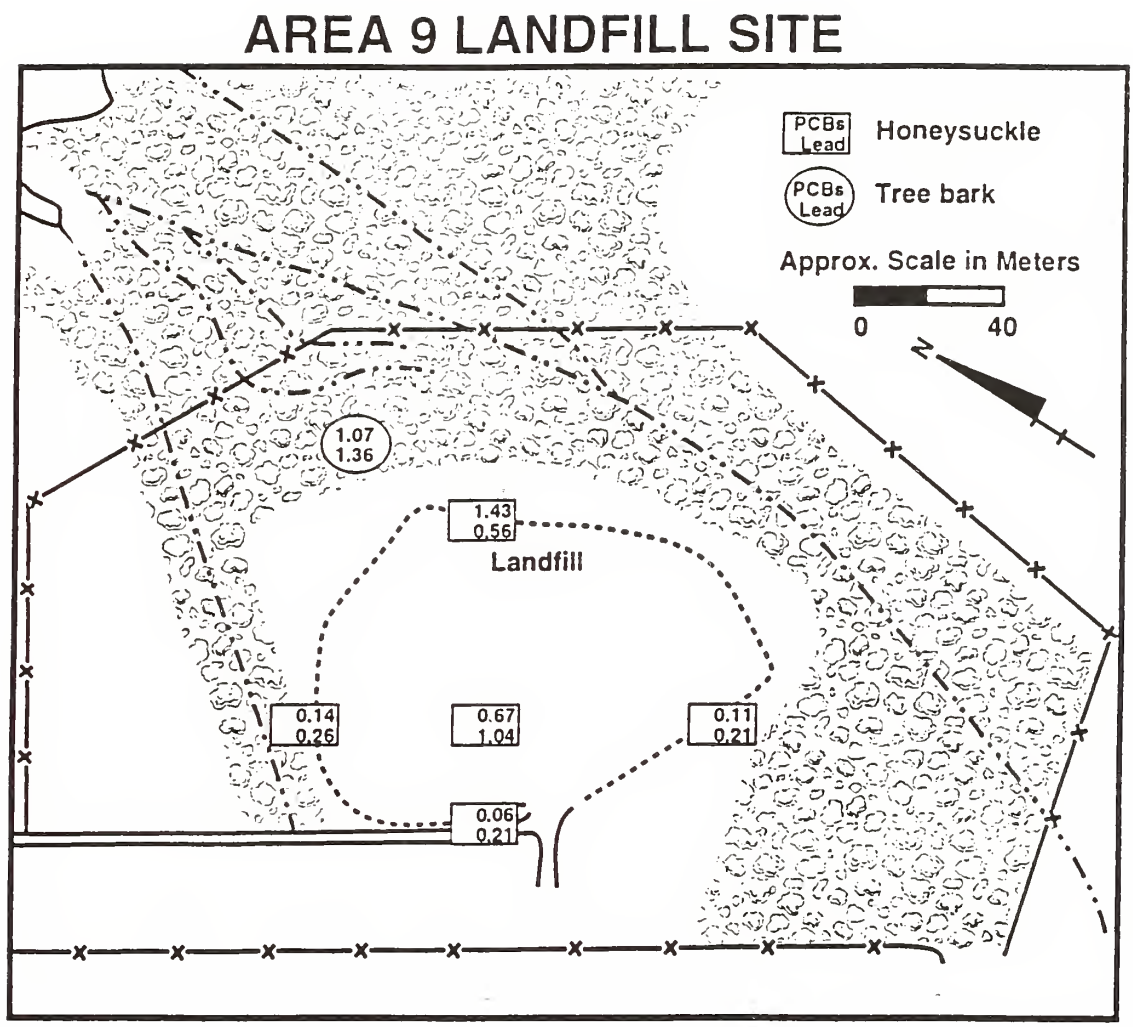

Figure 4. Concentration of PCBs as Aroclor 1254 (µg/g wet weight) and lead (µg/g dry weight) in tree bark and honeysuckle collected from Area 9 Landfill at Crab Orchard NWR.

In 1992, we restricted sampling to Area 9 Landfill and measured contaminant levels in beetles collected at several distances away from the landfill. Two species, *P. fervida* and *P. bipartita*, were collected in sufficient numbers to analyze. Aroclor 1254 was observed at the highest concentrations in beetles taken in light traps located directly on the landfill (Table 6). PCBs decreased rapidly as distance increased, however, detectable levels of PCBs were measured in the composite samples collected 500 m from the landfill.

Composite samples were necessary to provide enough tissue for duplicate PCB analysis, lead analysis, and moisture determinations. To ascertain variation among individuals, ten single adult beetles (0.4 to 1 g) for each species were analyzed for PCBs without duplicate or moisture analyses. Beetles for the individual analyses were taken from the trap located directly on Area 9 Landfill. The Aroclor 1254 concentration ranged from 0.77 to 18.1 µg/g for *P. fervida* and from 2.2 to 22.9 µg/g for *P. bipartita*. The mean (standard deviation) for this analysis was 9.5 (6.4) µg PCBs/g for *P. fervida* and 10.4 (7.4) µg PCBs/g for *P. bipartita*. Coefficients of variation for the ten individual beetles were 67.4% and 71.1% for *P. fervida* and *P. bipartita*, respectively. Despite high variation in total PCB concentrations, the congener profile was similar among individuals and not profoundly different from soil (Figure 3).

Lead was found in all beetle samples analyzed (Table 6). In contrast to PCBs, lead concentration in beetles did not vary with distance from the landfill (Table 6).

Table 5. Concentration of cadmium and PCBs (as Aroclor 1254) in *Phyllophaga fervida* collected from several sites at Crab Orchard NWR in 1991.

Site	Lipid, %	Body burden, µg/g wet weight[a]	
		Cadmium	PCBs
Reference site (Olin)	1.66	ND	ND
Old Refuge Shop	NM	ND	ND
Job Corp Landfill	1.47	NM	1.50[b]
Area 9 Landfill	3.06	NM	2.23[b]

[a] ND = not detected. NM = not measured.

[b] Average of two analytical replicates of single sample comprising from 20 to 30 g of beetles. Detection limits were 0.05 µg/g for cadmium and 0.01 µg/g for PCBs.

Table 6. Concentration of Aroclor 1254 and lead in *Phyllophaga fervida* and *P. bipartita* collected by light traps at various distances from Area 9 Landfill at Crab Orchard NWR in 1992.

		Distance from Area 9 Landfill[a]		
Species	Type of Measurement	0 m	50 m	500 m
P. fervida	% moisture	72.9 (1.1)	72.5 (1.0)	73.8 (1.9)
	lead (µg/g)	0.34 (0.08)	0.25 (0.11)	0.43 (0.48)
	Aroclor 1254 (µg/g)	10.60 (4.40)	1.24 (0.63)	0.30 (0.25)
P. bipartita	% moisture	73.0 (0.6)	72.9 (1.1)	74.0 (1.1)
	lead (µg/g)	0.24 (0.07)	0.56 (0.06)	0.56 (0.06)
	Aroclor 1254 (µg/g)	8.29 (1.94)	2.59 (1.56)	0.10 (0.01)

[a] Mean and standard deviations for five composite samples. Each sample was a composite of about 10 g of beetles. The detection limits were 0.05 µg/g dry weight for lead and 0.01 µg/g wet weight for Aroclor 1254.

Earthworms

Mortality of earthworms, *Eisenia foetida*, related well to the concentration of Aroclor 1254 in the soil (Table 7). Soil samples with measured Aroclor 1254 concentrations of 2,900 and 3,400 µg/g caused 100% mortality in test worms. The positive control containing 300 ppm of Aroclor 1254 yielded mortality of 37.5%. Mean weight of individuals was not significantly different between worms exposed to reference site soil (Olin) and the Area 9 Landfill sample with the lowest Aroclor 1254 concentration (0.5 ppm) (Table 7). Worms exposed to the positive control (300 ppm) had a significant reduction in weight compared to control.

Honey bees

In 1990, honey bees were collected from the brood chamber at the reference site (Olin), the Old Refuge Shop, and Area 9 Landfill. Composite samples of brood honey bees were not found to have detectable levels of cadmium or Aroclor 1254 (Table 8). Lead was identified in all brood bee samples, but the level was not significantly affected by the location of the hive (Table 8).

Forager bees returning to the hives from foraging trips were collected in 1991 and 1992. No attempts were made to dislodge pollen or other material from the surface of the bees. As observed for the brood bees, no Aroclor 1254 could be detected in forager bees (Table 8). In 1991, we observed higher levels of lead in forager bees collected at Area 9 Landfill compared to the Old Refuge Shop or the Olin reference site (Table 8). These results were unexpected, so we repeated the forager bee sampling in 1992 and found that the results were repeatable (Table 8).

Table 7. Growth and survival of the earthworm, *Eisenia foetida*, exposed in the laboratory to PCB contaminated soil collected from Area 9 Landfill at Crab Orchard NWR.

Treatment	Measured PCB Concentration	% Mortality		Group weight (mg)[a]		
		Day 7	Day 14	Day 0	Day 7	Day 14
Reference site	< 0.05	0.0 (0.0)	0.0 (0.0)	487.5 (22.4)	437.1 (21.9)	373.7 (7.4)
HWS-1	2,925	100.0 (0.0)	100.0 (0.0)	489.9 (29.1)	NA	NA
HWS-2	0.5	0.0 (0.0)	0.0 (0.0)	475.5 (24.2)	417.4 (22.5)	366.8 (25.9)
HWS-3	645	29.2 (0.1)	100.0 (0.0)	470.7 (23.2)	295.6 (7.2)	NA
HWS-4	3,408	100.0 (0.0)	100.0 (0.0)	468.2 (36.9)	NA	NA
Positive control	300	0.0 (0.0)	37.5 (0.0)	481.3 (32.0)	361.8 (14.3)	262.9 (23.0)

[a] NA = not applicable. All worms were dead in these samples.

Table 8. Concentration of cadmium, lead, and Aroclor 1254 in brood and forager honey bees collected from several sites at Crab Orchard NWR.

Site	Sample Type	Year Collected	n	Moisture (%)	Lipid (%)	Body burden, µg/g wet weight[ab] Cadmium	Lead	Aroclor 1254
Reference site (Olin)	Brood	1990	3	71.2 (0.1)	2.3 (0.0)	ND	0.49 (0.06)	ND
	Forage	1991	3	70.2	1.6	NM	0.49 (0.06)	ND
	Forager	1992	5	73.1 (0.6)	NM	NM	0.26 (0.12)	ND
Old Refuge Shop	Brood	1990	3	69.7 (0.2)	3.0 (0.1)	ND	0.69 (0.39)	ND
	Forager	1991	3	72.2	1.4	NM	0.61	ND
	Forager	1992	5	72.1 (0.2)	NM	NM	0.26 (0.07)	ND
Area 9 Landfill	Brood	1990	3	74.8 (0.2)	3.1 (0.2)	ND	0.55 (0.03)	ND
	Forager	1991	3	73.1	1.3	NM	1.36 (0.10)*	ND
	Forager	1992	5	72.2 (0.7)	NM	NM	2.08 (0.33)*	ND

[a] Mean and standard deviation, n = 3. One sample was a single homogenate of 30 g of honey bees in 1990. In 1991 and 1992 one sample was a single homogenate of 10 g of bees. ND = not detected. NM = not measured. The detection limits were 0.05 µg/g dry weight for lead and 0.01 µg/g wet weight for Aroclor 1254.

[b] * denotes significantly different from reference site using one-way Anova and Tukey's multiple means comparison test (p=0.05).

White-footed mice

A total of 71 mice were trapped at the three study sites (Table 9). Trapping success, a measure of catch per unit effort, was not different between the Area 9 Landfill and reference site (Area 13), but slightly lower at the Job Corp Landfill. Males composed 54% of the total adult catch at Area 9 Landfill and 44% for the Job Corp site compared to 50% for the control site. Combined catches of subadult and juvenile mice expressed as percentage of total catch was 10.7% for Area 9 Landfill, 6.3% for the Job Corp Site, and 7.7% for the control site. Since juveniles and subadults were a small proportion of total catch, these animals were not included in morphometric and biochemical analysis.

Table 9. Trapping success for white-footed mice collected from a reference site (Area 13) and several hazardous waste sites at Crab Orchard NWR.

		Number mice trapped[a]						
	Total[b]	Female			Male			Trapping[c]
Site	trap night	A	S	J	A	S	J	success
Reference site	501	13	0	0	11	1	1	0.052
Job Corp Landfill	501	8	1	0	8	0	0	0.034
Area 9 Landfill	501	11	1	0	14	1	1	0.056

[a] A=adult; S=subadult; J=juvenile.

[b] Represents the total of three nights for each of 167 traps.

[c] Trapping success= $\frac{\text{Total number trapped}}{\text{Total trap nights}}$

Soil concentrations of Aroclor 1254 along transects at Area 9 Landfill were highest on the landfill proper (Figure 2). Carcass concentrations of Aroclor 1254 in mice collected along these same transects are shown in Figure 5. In general, mouse carcass concentrations tended to be highest in animals captured directly on the landfill, however, some exceptions occurred. For example, a male mouse captured at location 25 on transect 2, about 50 m off the landfill, had residues of 25.2 µg/g wet weight.

Mice collected at Area 9 and Job Corp landfills had significantly higher Aroclor 1254 body burdens than mice collected at the reference site (Table 10). Analysis of the eight most abundant congeners indicates no differences in PCB profiles between mice collected at Area 9 Landfill compared to Job Corp Landfill (Table 11). The 2,2',4,4',5,5'-hexachlorobiphenyl congener (IUPAC 153) was the most abundant congener in mice tissues followed by PCB congeners 138 and 180. No simple relationship relating the degree and position of chlorination with percent composition was apparent. Total PCBs, calculated by summing concentrations of the eight select PCB congeners, accounted for most of the PCB body burdens when compared, weight to weight, with body burdens expressed as Aroclor 1254. The significance of this observation is that the eight congeners account for the bulk of PCB residues.

High PCB body burdens at the Area 9 Landfill were associated with elevated relative liver weight and monooxygenase activity (Table 10). Mice from the Job Corp Landfill followed a similar trend, but were not significantly different from control animals. No significant differences were observed in the relative tissue weight of testes, epididymis, uteri, ovaries, or adrenals (Table 12). Relative kidney weights in males from the Job Corp Landfill were significantly lower compared to animals from the reference site

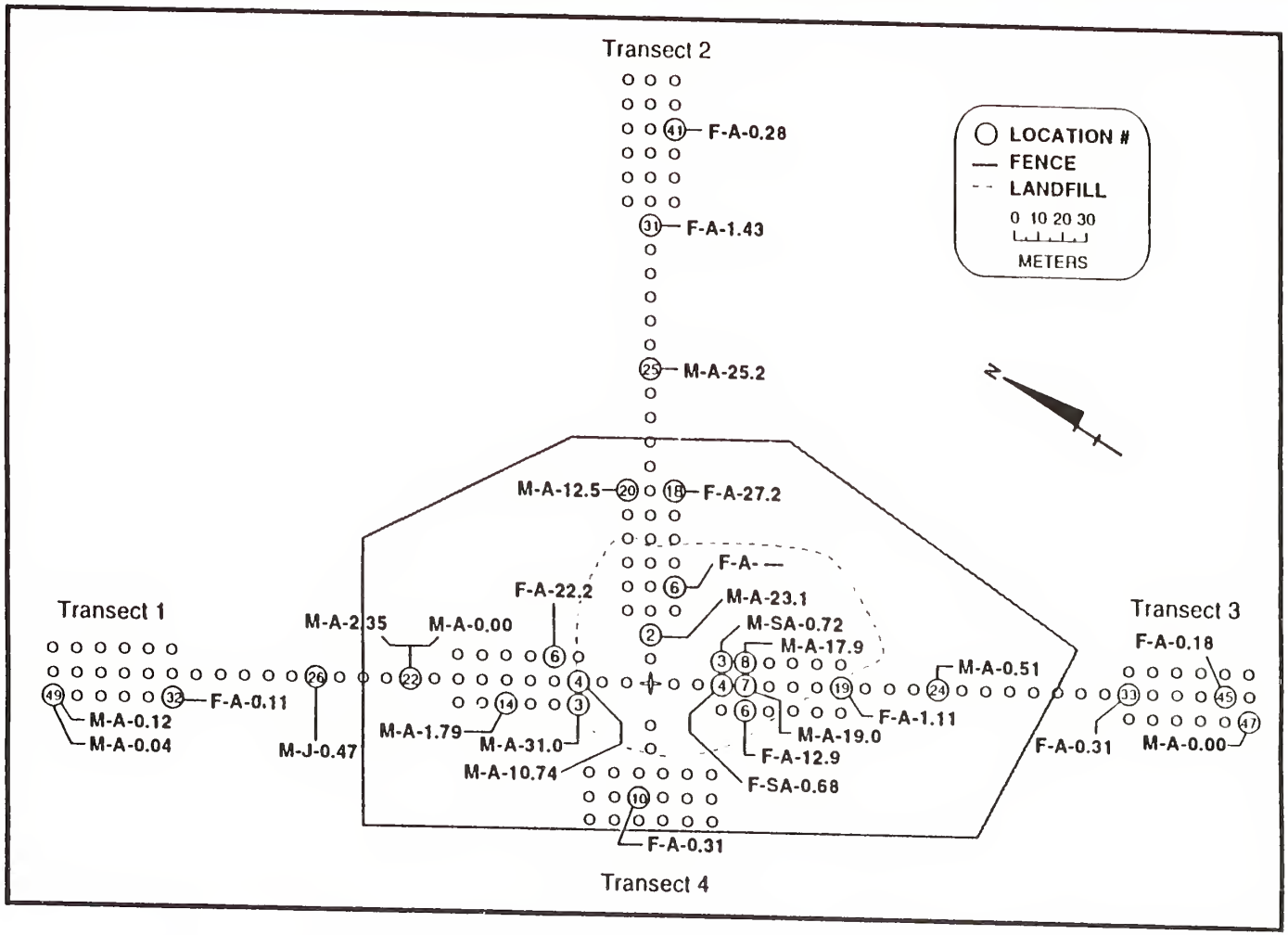

Figure 5. Concentration of PCBs as Aroclor 1254 in *Peromyscus leucopus* collected at Area 9 Landfill. Code: Sex (M=male, F=female); Age (A=adult, SA=subadult, J=juvenile); PCB concentration (µg/g wet weight). Open circles indicate location of unsuccessful traps.

Table 10. Body weight, percent lipid, relative liver weight, EROD activity, PROD activity, and PCB concentration in adult white-footed mice collected from a reference site (Area 13) and several hazardous waste sites at Crab Orchard NWR.

			Mean and Standard Deviation [a]					
Site	Sex	n	Body weight (g)	Aroclor 1254 µg/g (wet weight)[b]	Aroclor 1254 µg/g (lipid weight)	Relative liver weight, %[c]	EROD[d]	PROD[d]
Reference site	F	13	23.1 (4.0)	0.01 (0.03)	0.45 (1.14)	4.37 (0.47)	0.05 (0.04)	0.01 (0.00)
	M	11	21.0 (2.3)	0.01 (0.03)	0.45 (1.00)	4.40 (0.76)	0.07 (0.06)	0.01 (0.00)
Job Corp	F	8	23.3 (3.3)	2.21 (3.44)	162.9 (225.9)	5.05 (0.79)	0.31 (0.34)	0.01 (0.01)
Landfill	M	8	22.3 (1.6)	0.78 (1.23)	94.6 (157.1)	4.08 (0.67)	0.20 (0.27)	0.01 (0.01)
Area 9 Landfill	F	11	22.5 (3.9)	6.6 (10.4)*	323.4 (442.5)*	6.65 (3.26)*	0.46 (0.60)*	0.02 (0.02)*
	M	14	23.0 (1.9)	10.3 (11.1)*	843.4 (1,032)*	6.76 (2.16)*	0.59 (0.48)*	0.02 (0.01)*

[a] * denotes significant difference from mice of the same sex collected at the reference site ($p<0.05$) using one-way ANOVA and Tukey's multiple means comparison ($p=0.05$).

[b] Detection limit for Aroclor 1254 was 0.01 µg/g wet weight.

[c] Calculated as $\frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$

[d] Expressed as pmol resorufin formed/mg microsomal protein/min.

(Area 13), whereas females from the Job Corp Landfill were not significantly affected. The opposite was observed for relative spleen weights where females were affected but males were not (Table 12). Lipid content (Table 12) was not different among sites, but was significantly different between sexes. Females had higher lipid content than males as determined using a two-way ANOVA with sex and site as the main effect means.

Data from all sites were pooled and correlation analysis was used to investigate associations between several different variables (Table 13). Significant correlation coefficients were observed for females between body weight and relative kidney weight, relative adrenal weight, relative uterus weight, and percent lipid. For males, the relative testes weight was the only tissue correlated to body weight.

Total PCBs, expressed relative to wet weight or lipid content, were significantly correlated to relative liver weight, EROD, and PROD activity in both females and males (Table 13). The nature of the correlation is shown in Figure 6 for relative liver weight, Figure 7 for EROD activity, and Figure 8 for PROD activity. For females, expressing PCBs relative to lipid content reduced the correlation coefficient with relative liver weight, but improved the correlation coefficient with the monooxygenase enzymes. Similar effects were not observed for males. Lipid content (%) of females, but not males, was significantly correlated with total PCBs.

Table 11. PCB congener composition in white-footed mice collected from hazardous waste sites at Crab Orchard NWR.

PCB congener[b]	Job Corp Landfill[a] Percent composition	Area 9 Landfill[a] Percent composition
99 (2,2',4,4',5)	7.05 (3.21)	6.54 (3.01)
105 (2,3,3',4,4')	1.35 (0.99)	2.76 (2.08)
118 (2,3',4,4',5)	0.34 (0.25)	0.56 (0.29)
138 (2,2',3,4,4',5')	16.99 (2.91)	17.86 (4.14)
153 (2,2',4,4',5,5')	47.65 (2.04)	45.81 (6.07)
170 (2,2',3,3',4,4',5)	7.84 (1.68)	7.76 (1.92)
171 (2,2',3,3',4,4',6)	1.04 (0.17)	1.62 (0.66)
180 (2,2',3,4,4'5,5')	17.73 (6.54)	17.09 (6.87)
Total PCBs (µg/g)[c]	4.62 (3.10)	13.31 (10.32)
% of Aroclor 1254[d]	110.0 (22.8)	96.5 (18.3)

[a] Percent composition is the percentage of one particular congener relative to the total of the eight congeners appearing in the table. n=5 for the Job Corp site and n=13 for the Area 9 Landfill.

[b] PCB congeners designated using the IUPAC numbers according to Ballschmitter and Zell (1980). Chlorine substitution shown in parentheses.

[c] Total PCBs is the summation of the concentration of each congener, not the summation of the percentages.

[d] % of Aroclor 1254 is calculated by comparing total PCBs (see footnote c) with the independent determination of Aroclor 1254 (see material and methods) as follows:

$$\% \text{ of Aroclor 1254} = \frac{\text{Total PCBs (µg/g)}}{\text{Aroclor 1254 (µg/g)}} \times 100$$

Table 12. Relative tissue weight for kidney, adrenal, spleen, gonads, and lipid from adult white-footed mice collected from a reference site (Area 13) and hazardous waste sites at Crab Orchard NWR.

			Relative tissue weight, % [a]					
Site	Sex	n	Kidney	Adrenal	Spleen	Ovary or Testes	Uterus or Epididymis	Lipid, %
Reference site	F	13	1.59 (0.26)	0.09 (0.02)	0.28 (0.14)	0.16 (0.21)	7.01 (8.22)	1.83 (0.67)
	M	11	1.54 (0.15)	0.08 (0.02)	0.56 (0.69)	2.21 (0.89)	0.80 (0.22)	1.44 (0.61)
Job Corp	F	8	1.39 (0.18)	0.07 (0.01)	0.52 (0.27)*	0.12 (0.04)	4.98 (5.99)	1.54 (0.49)
Landfill	M	8	1.35 (0.15)*	0.10 (0.02)	0.38 (0.28)	2.72 (0.23)	0.74 (0.28)	1.11 (0.22)
Area 9 Landfill	F	11	1.55 (0.29)	0.08 (0.02)	0.41 (0.19)	0.42 (0.30)	5.46 (8.30)	2.04 (0.89)
	M	14	1.48 (0.17)	0.08 (0.02)	0.42 (0.30)	2.68 (0.34)	0.72 (0.15)	1.25 (0.53)

[a] * denotes significant difference from mice of the same sex collected at the reference site ($p<0.05$) using one-way ANOVA and Tukey's multiple means comparison test ($p=0.05$).

Table 13. Correlation of PCB concentration in white-footed mice with lipid, relative liver weight, and monooxygenase activity.

		Correlation Coefficient[a]		
Variable	Sex	Body Weight	Total PCB (wet weight)	Total PCB (lipid)
Body weight	Female	--	-0.04	-0.14
Liver weight[b]		-0.04	0.93 ***	0.74 ***
Kidney weight[b]		0.55 ***	0.12	0.15
Adrenal weight[b]		-0.65 ***	-0.13	-0.09
Spleen weight[b]		0.08	0.07	-0.06
Ovaries weight[b]		0.01	-0.10	-0.12
Uteri weight[b]		0.60 ***	-0.19	-0.16
Lipid, %		-0.50 **	0.52 ***	0.21
EROD		-0.15	0.74 ***	0.84 ***
PROD		-0.05	0.75 **	0.87 ***
Body weight	Male	--	-0.11	0.16
Liver weight[b]		0.33	0.84 ***	0.80 ***
Kidney weight[b]		-0.01	0.12	0.20
Adrenal weight[b]		0.16	-0.18	-0.28
Spleen weight[b]		-0.06	-0.13	-0.11
Testes weight[b]		0.40 *	0.20	0.22
Epididymis weight[b]		0.15	0.07	0.06
Lipid, %		0.15	-0.07	-0.27
EROD		0.06	0.65 ***	0.72 ***
PROD		0.15	0.42 *	0.55 ***

[a] * denotes significant correlation coefficient. * = 0.05 > P > 0.01; ** = 0.01> P > 0.001; and *** = P < 0.001.

[b] Correlation performed on relative tissue weights (expressed as % of total body weight).

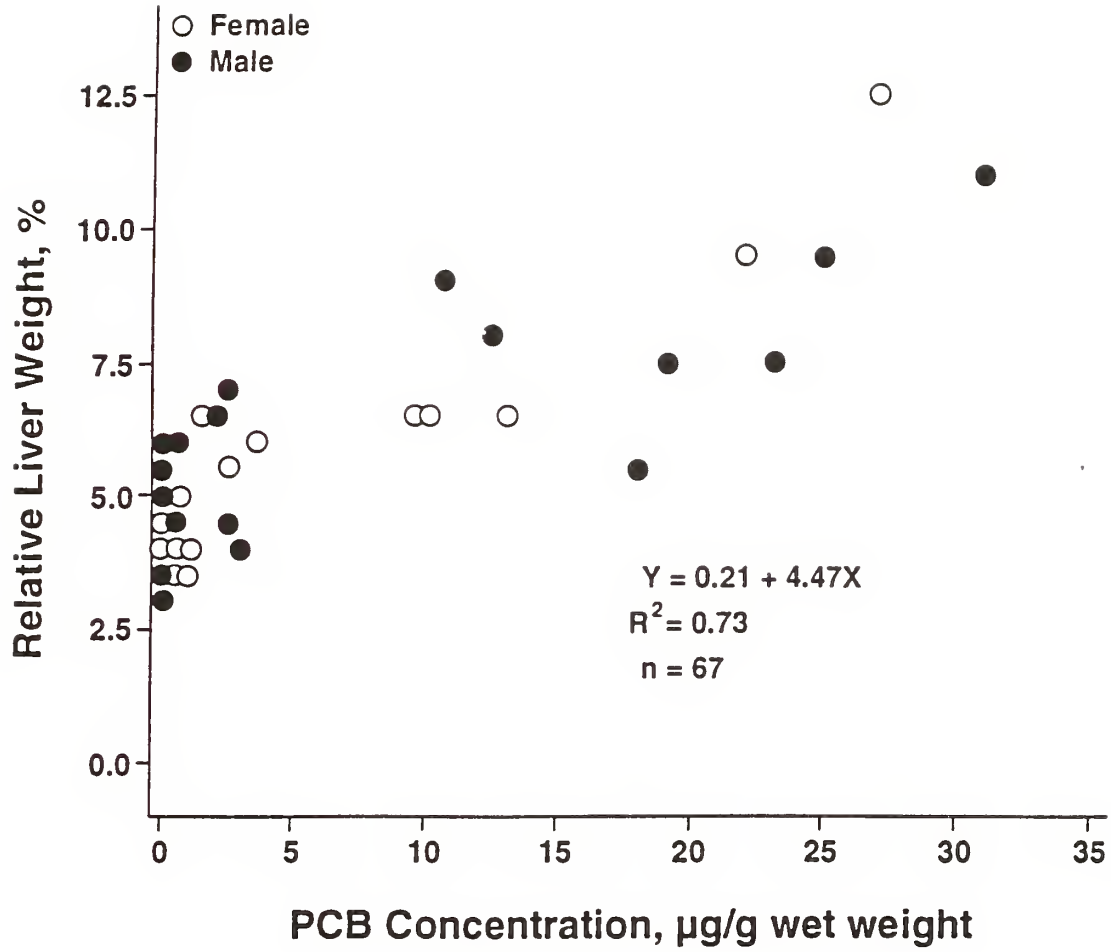

Figure 6. Regression parameters and plot for relative liver weight (%) versus PCB concentration in *Peromyscus leucopus* collected at Crab Orchard NWR.

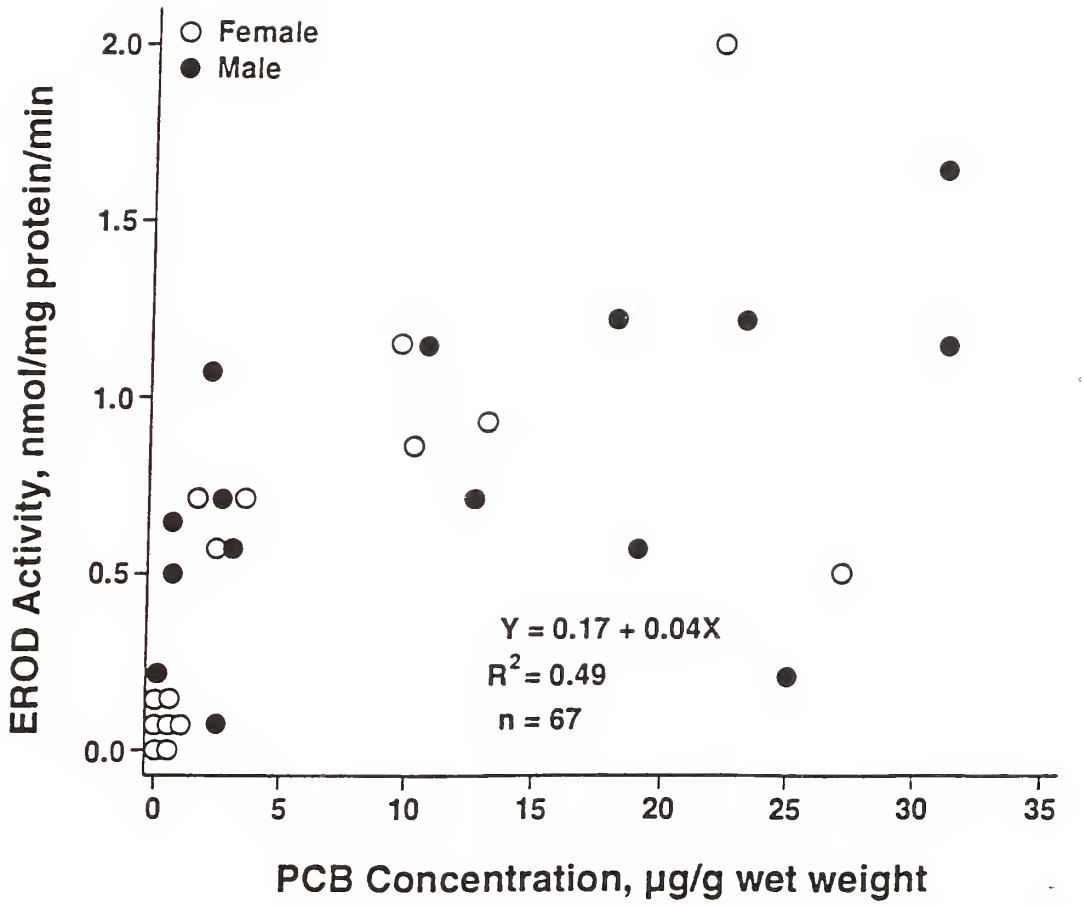

Figure 7. Regression parameters and plot for ethoxyresorufin-O-dealkylase (EROD) activity versus PCB concentration in *Peromyscus leucopus* collected at Crab Orchard NWR.

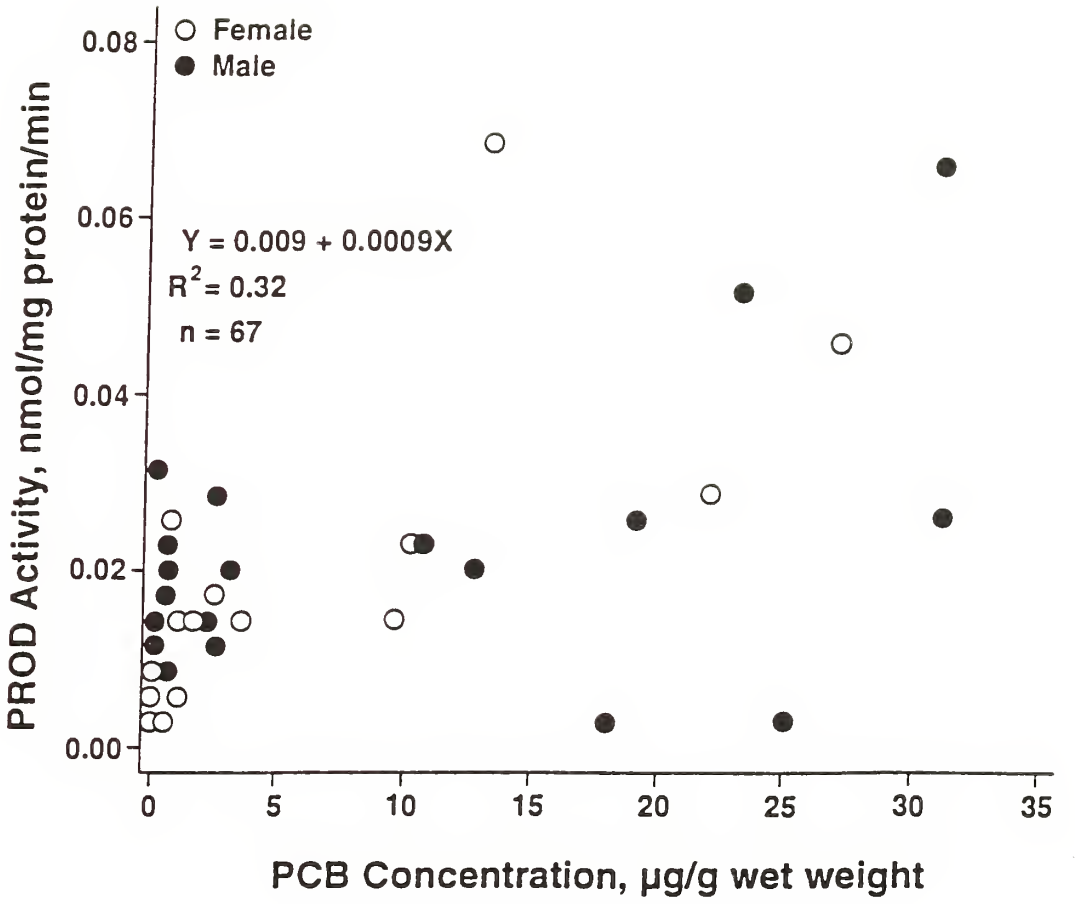

Figure 8. Regression parameters and plot for pentoxyresorufin-O-dealkylase (PROD) activity versus PCB concentration in *Peromyscus leucopus* collected at Crab Orchard NWR.

The nature of the association between PCB body burden and relative liver weight, EROD activity, and PROD activity was further investigated using regression analysis. Linear regression was initially calculated and although significant regression parameters were identified, significant deviation from linear regression was detected using the method described by Sokal and Rohlf (1969). The significant deviation from regression (sometimes referred to as lack of fit) can result from a high degree of heterogeneity in the data or from curvilinear tendencies in the data. Curvilinearity was investigated using the polynomial $y=a+bx+cx^2$ using SAS nonlinear procedures. Estimation of the regression parameters indicated strong nonlinear trends for EROD and PROD data, but not relative liver weight (Table 12). This trend is indicated by the magnitude of the coefficient, c, in the nonlinear regression. In the case of relative liver weight, c is not significantly different from 0. Under conditions where c=0 the cx^2 term drops out and only the linear portion remains (i.e. $y=a+bx$). Indeed, the values of the intercept (a) and slope (b) in Table 8 are similar to values estimated from linear regression analysis for relative liver weight (intercept=4.47 and slope=0.21, Figure 6). Consequently, deviation from regression for relative liver weight is most likely due to heterogeneity. For EROD and PROD activity, the coefficient c is significantly different from 0, indicating that a portion of the deviation from regression is due to nonlinearity in the data.

Table 14. Nonlinear regression analysis for relative liver weight, EROD activity and PROD activity as a function of carcass PCB concentration for white-footed mice collected at Crab Orchard NWR.

	Regression Coefficients[a]			
Variable	a	b	c	R^2
Relative liver weight	4.46 (0.14)	0.20 (0.06)	0.0001 (0.0023)	0.97
EROD activity	423.6 (110.5)	116.4 (14.2)	-2.07 (0.33)	0.90
PROD activity	7.4 (1.5)	2.5 (0.6)	-0.07 (0.02)	0.71

[a] Standard error in parentheses. Coefficients estimated by fitting the curvilinear polynomial $y = a + bx + cx^2$ using the iterative nonlinear procedure of SAS.

Starlings

Nine nestboxes were monitored for starling nesting activity at the reference site and 18 at Area 9 Landfill. The distribution of the nestboxes is shown on Map 1, Appendix I. Adult starlings began selecting nestboxes in March. Egg laying was initiated around the first of April and proceeded through early June. Some starling pairs produced two successful nests in one nesting season. About five days were required to complete a clutch and clutches usually had 4 or 5 eggs. Incubation generally lasted about 11 to 13 days and nestlings were collected at 15 days post-hatch, about two days before they would normally fledge. Several growth parameters and PCBs were measured in collected nestlings. The growth variables for nestlings from the reference site were analyzed for sexual dimorphism. The only growth parameter that showed significant difference between sexes was the length of the right tarsus (Table 15). Since sexual differences were small, data for different sexes was pooled within a particular experimental unit (i.e., reference, 10 m, 60 m, and 200 m units) to increase the statistical power.

Aroclor 1254 was not found in nestlings collected from the Olin reference site (Table 16). All nestlings collected from boxes on or near Area 9 Landfill had measurable residues of Aroclor 1254. The concentration of PCBs was highest in nestlings sampled from the boxes closest to the landfill and decreased as the distance increased (Table 16). The distribution of PCB levels among nestboxes is shown in Figure 9. The values presented for each nest box represents the mean and standard deviation of whole body residues for all nestlings collected from that box. The congener profile extracted from contaminated starlings closely resembled Aroclor 1254.

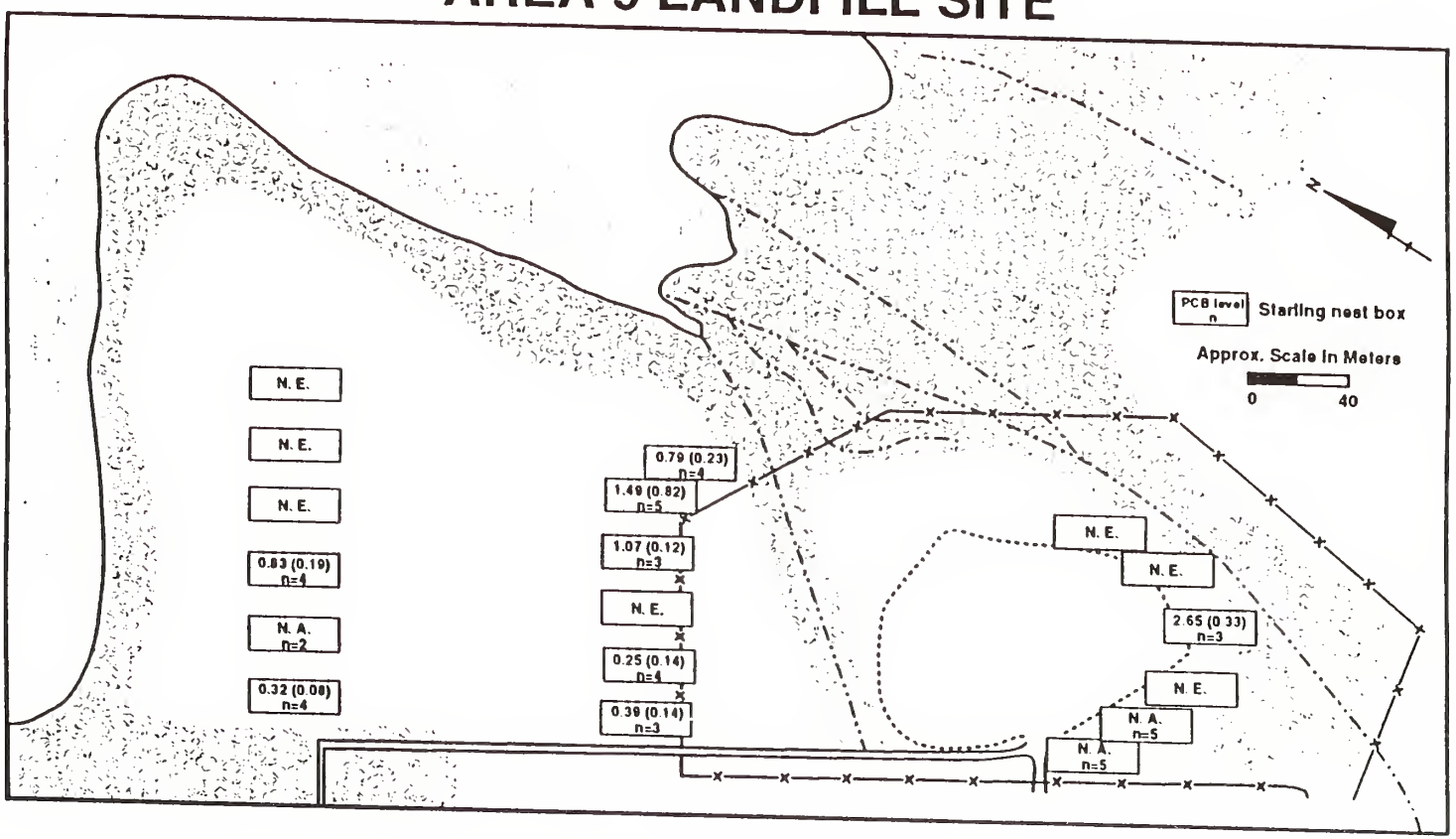

Figure 9. Concentration of PCBs as Aroclor 1254 (µg/g wet weight) in 15 day old starlings, *Sternus vulgaris* at various distances from Area 9 Landfill (concentrations presented as standard deviation for nestmates). N.E.=no eggs, N.A.=eggs abandoned.

Table 15. Comparison of morphometric data for 15 day old male and female starlings collected at the Olin reference site on Crab Orchard NWR.

	Mean and standard deviation[a]	
Variable	Female	Male
Sample size, n	20	19
Body weight, g	69.6 (7.1)	72.1 (6.2)
Relative liver weight, %	5.6 (1.1)	5.4 (0.9)
Wing length, mm	85.6 (6.6)	88.2 (4.6)
Primary feather (5th), mm	53.3 (6.0)	54.5 (4.7)
Sheath length, mm	18.1 (2.8)	17.9 (1.8)
Tarsus length, mm	29.1 (0.8)*	29.9 (0.8)*
Lipid content, %	3.7 (1.7)[b]	3.8 (1.9)[b]
Water content, %	72.3 (2.8)[b]	71.5 (2.0)[b]
Increase in mass, g (Day 3 to Day 9)	38.7 (7.6)	41.1 (6.7)

[a] Units for means given in the variable list. * denotes significant difference between sexes using ANOVA and Tukey's multiple means comparison test (p=0.05).

[b] n=14 for females and n=16 for males.

Table 16. Comparison of morphometric data for 15 day old starlings collected at the Olin reference site and at several distances from Area 9 Landfill at Crab Orchard NWR.

	Mean and Standard Deviation [a]			
		Area 9 Landfill		
Site	Reference	200 m	60 m	10 m
Sample size, n	39	8	20	3
PCB concentration	ND	0.57 (0.30)	0.84 (0.63)	2.66 (0.33)
Body weight, g	70.8 (6.7)	70.1 (7.1)	70.4 (5.2)	66.1 (4.7)
Rel. liver wt., %	5.5 (1.0)	5.2 (1.0)	5.1 (1.1)	6.5 (0.3)
Wing length, mm	86.8 (5.8)	88.4 (2.5)	88.1 (6.2)	75.7 (4.6)*
Primary sheath, mm	53.9 (5.4)	56.1 (2.2)	54.9 (4.9)	42.3 (5.1)*
Sheath length, mm	18.0 (2.3)	17.0 (1.7)	17.3 (2.0)	16.3 (3.1)
Tarsus length, mm	29.5 (0.9)	29.0 (0.7)	29.4 (0.6)	27.2 (0.9)*
Lipid, %	37.8 (18.0)	48.0 (27.5)	43.6 (35.1)	17.0 (6.4)
Water, %	71.8 (2.4)	70.2 (3.0)	70.7 (3.1)	74.9 (1.2)
Increase in mass (Day 3 to 9)	38.1 (11.7)	42.7 (3.0)	39.9 (3.7)	34.4 (4.6)

[a] * denotes significant difference from nestling collected at the reference site ($p<0.05$) using one-way ANOVA and Tukey's multiple means comparison. Units for mean are given in the variable list.

Nestlings collected nearest the landfill were siblings and had significant reductions in wing length, primary sheath length, and tarsus length compared to nestlings collected at the Olin reference site (Table 16). These changes in growth parameters were associated with decreased lipid reserves (% lipid content) and increased liver weights, although the changes in these variables were not statistically significant. The only parameter that was correlated with carcass PCB levels was tarsus length (Table 17). The negative correlation indicates that increased PCB concentrations are associated with reduced tarsal growth. Standardizing PCB body burdens to lipid content improved the correlation with tarsus length, but did not identify any other significant relationships. Low level contamination (i.e. less than 1 mg/kg) was not associated with reductions in relative liver weight or any of the growth parameters measured (Table 17).

Occupancy rates of nestboxes, as evidenced by the number of boxes occupied and number of eggs laid, was not different between the control and the hazardous waste site. Eleven nests were initiated at both the reference site and the Area 9 Landfill (Table 18). Moreover, these 11 nests were associated with production of 54 eggs at both sites (Table 18).

Percent hatch of eggs in nestboxes adjacent to the Landfill was only 22.2% compared to a minimum of 97.2% at the other sites (Table 18). The low nestling yield was not caused by the absence of egg laying in these boxes. Rather, the low yield was caused by low hatching success. Unsuccessful hatching was observed in two clutches of eggs laid in the boxes nearest the landfill. Complete failure to hatch was not observed in any of the other nest boxes. Eggs were allowed to remain in the nest boxes until 15 days after the clutch was completed, even if we suspected that incubation had been terminated. This was necessary because incubation by

Table 17. Correlation of PCB concentration with lipid, relative liver weight, and growth variables in starlings collected from several locations at Area 9 Landfill at Crab Orchard NWR.

	Correlation Coefficient[a]		
Variable	Body Weight	Total PCB (wet weight)	Total PCB (lipid)
Body weight	--	0.04	-0.10
Liver weight[b]	-0.54***	0.01	0.14
Lipid, %	0.60***	0.18	-0.25
Tarsus	0.60***	-0.33**	-0.42**
Wing length	0.51***	-0.19	-0.23
Primary feather	0.51***	-0.21	-0.28
Primary sheath	-0.16	-0.16	-0.19
Increase in mass	0.33**	0.06	0.02

[a] * denotes correlation coefficient significantly different from zero at $0.05 > p > 0.01$; ** denotes significant difference at $0.01 > p > 0.001$; *** denotes significance at $p < 0.001$.

[b] Correlation performed on relative tissue weights (expressed as % of total body weight).

Table 18. Occupancy, hatching, and fledging success for starling nestboxes located at the Olin reference site and Area 9 Landfill at Crab Orchard NWR.

Site	Number of Nest Boxes	Number of Nests [a]	Number of Eggs	Number Hatched	Hatch%[b]	Number Fledged	Fledged%[b]
Reference Site (Olin)	9	11	54	53	98.2(6.0)	46	85.5(15.7)
Area 9 Landfill - 200m	6	2	8	8	100.0 [c]	8	100.0 [c]
Area 9 Landfill - 60m	6	6	30	29	97.2(6.8)	20	69.5(36.8)
Area 9 Landfill - 10m	6	3	16	4	22.2(38.5)	3	75.0 [d]

[a] Nest is defined as nest box activity resulting in clutch with greater than 3 eggs.

[b] Mean and standard deviation. Individual nests served as the error term.

[c] Average of 2 observations.

[d] Fledging calculation based on only one nest since 2 nests had no hatch.

adults was frequently erratic for these nests and it was not certain that the eggs had been abandoned. At day 15, eggs not pipped were collected and returned to the laboratory. The eggs were opened to determine the stage of development. It was clear that the eggs had been viable, but development had stopped at an early stage of development (3 to 5 days). Nestling survival after hatch was not different among the different sites (Table 18).

Canada geese

Aroclor 1254 was observed in subcutaneous fat samples in 38% (3 of 8) of the geese collected at Job Corp Landfill and Area 9 Landfill (Table 19). The concentration was not high and was variable among samples. Aroclor 1254 was not detected in breast muscle tissue. Lead was measured in breast muscle tissue, was not elevated above background levels, and was not different between the two sites. The high coefficient of variation for lead samples at the Job Corp Landfill was a result of one sample that had a relatively high level of lead (7.0 µg/g).

Table 19. Concentration of Aroclor 1254 and lead in breast and subcutaneous fat of Canada geese collected from the Job Corp Landfill and the Area 9 Landfill at Crab Orchard NWR.

			Hazardous Waste Site[a]			
			Job Corp Landfill		Area 9 Landfill	
Tissue Type	Contaminant[b]	n	Number with Contaminant	Mean (SD)	Number with Contaminant	Mean (SD)
Breast	Lead	8	8	0.50 (0.08)	8	1.25 (2.28)
	Aroclor 1254	8		ND		ND
Fat (subcutaneous)	Lead	8		NM		NM
	Aroclor 1254	8	3	1.32 (1.95)	3	0.41 (0.20)

[a] ND= Not detected (below detection limits of 0.01 µg/g). NM= Not measured.

[b] Lead concentrations expressed as µg/g dry weight. PCB concentration expressed as µg/g wet weight.

DISCUSSION

Plants

Tree bark was selected as a monitor of PCBs because it may provide a relatively easy method for measuring remediation success. Bark tissues collected from pin oak trees adjacent to Area 9 Landfill have current levels of around 1 µg/g wet weight PCBs. Honeysuckle levels were variable, but also reached the 1 µg/g level of PCBs. These levels are considerably higher than background values for foliage (Buckley, 1988) and tree bark (Hermanson and Hites, 1990). Oak trees remaining near Area 9 Landfill after the remediation could be used to track post-remediation decontamination.

The highest level of lead observed in honeysuckle foliage was 1 µg/g dry weight and was collected near the center of the landfill where soil lead levels ranged from 360 to 2870 µg/g. Ruelle (1983a) reported similar levels in honeysuckle foliage from Area 9 Landfill with a maximum value of 0.46 µg/g. Eisler (1988) reported background levels of lead in terrestrial plants to be below 50 µg/g, in general, and values in excess of this can represent contamination by smelters or roadways. The low levels of lead observed in honeysuckle foliage at Area 9 Landfill are not surprising since the source of lead is the soil and because soil lead does not tend to volatilize, be easily taken up by roots except at relatively low pHs, or be effectively translocated even if it is absorbed (Demayo *et al.*, 1982).

Honeysuckle was investigated in this study in an attempt to identify a route by which herbivores could be exposed to lead. Woolf *et al.* (1983) reported elevated levels of lead in livers from white-tailed deer at Crab Orchard NWR. Ruelle (1984) reported elevated levels of several metals including lead in a doe collected near Area 9 Landfill. Data presented in this study and by Ruelle (1983b, 1984) suggests that contaminated plant

foliage on or near lead contaminated landfill is unlikely to be the route for transfer to white-tailed deer. The source of lead and the route of uptake by deer remains unknown at present.

Terrestrial invertebrates

Lead is a natural element present in many ecosystems. Background levels in biota from this study (i.e. 0.13 to 0.69 ppm wet weight or 0.43 to 3.1 ppm dry weight) are similar to background levels from other studies (Eisler, 1988). Biological transfer of environmental lead is principally via ingestion (Talmage and Walton, 1991). Consequently, the concentration of lead in plant material can determine body burden levels in primary consumers. Lead uptake by plants is dependent on soil moisture, organic carbon content, and pH (Demayo *et al.*, 1982). Since these parameter of soil can vary substantially, it is difficult to generalize about concentration factors for plants grown in contaminated soils. However, lead concentration in plants tends to be lower than "bulk" concentration of lead in the soil (Demayo *et al.*, 1982). This is supported by findings in this study where lead concentrations were several orders of magnitude lower than in the soil.

Similarly, concentration factors for invertebrates feeding on plants and detritus, calculated by dividing the concentration of a metal in an organism by the concentration of the metal in the diet (Hopkin, 1989), are generally between 0 and 1 (Connell and Miller, 1984; Hopkin, 1989). This observation suggests a low propensity of lead in soil to accumulate through food chains. However, even with low concentration factors, we would expect to see elevated lead concentration in soil fauna from areas with highly contaminated soil. For example, Johnson *et al.* (1978) found lead concentrations averaging 62 ppm dry weight in invertebrates collected from an area with soil lead levels of about 14,000 ppm dry weight. Thus, relatively high invertebrate lead levels were achieved despite low concentration factors.

No lead accumulation was observed at Area 9 Landfill for June beetles in the present study or for caged house crickets reported elsewhere (McKee, 1992). The lack of accumulation in house crickets was not surprising since crickets were caged and not allowed to forage on "natural" food from the contaminated area. For June beetles adults, however, it seemed likely that high levels of lead would be observed, since the beetle grubs live in soil and we observed high levels of PCBs in beetles. The absence of lead accumulation in June beetle adults may be attributable to several factors: 1) June beetle grubs may not readily absorb lead; 2) lead was released in the exuviae during the pupal molt to the adult life form; and 3) "pooling" of individuals may have decreased ability to detect increase in lead content of some individuals. Elimination of metals in molted exoskeletons has not been examined in June beetles, but this method has not been found highly effective in other invertebrate species (Hopkin, 1989). As mentioned earlier, other soil invertebrates, such as earthworms, typically have concentration factors, relative to soil, of less than one (Hopkin, 1989). Therefore, the lack of elevated lead levels is probably a result of low accumulation and pooling of individuals.

Honey bee foragers at Area 9 Landfill were the only organisms found to bioaccumulate lead in this study, however, the source and route of exposure remains unclear. Bee uptake of lead from ambient air is unlikely, since other insect species investigated were exposed to similar air concentrations. Infrequent contact of bees with soil makes it unlikely that lead is moving directly from soil to bees. The most plausible explanation at present is exposure of bees to lead contaminated plant material. Contamination of plant tissues by lead is dependent on the source of lead. Deposition of lead from polluted air is the major route of plant contamination as evidenced by the number of reports in the literature (Eisler, 1988). However, in landfill situations, relatively little lead becomes airborne and the main source of plant contamination is root uptake and translocation. Since lead is

relatively poorly translocated from the soil to upper plant tissue (Demayo *et al.*, 1982), exposure via this route should be low. Initial investigations of plant contamination at the site have failed to identify the particular exposure route for the forager bees. Other investigators have found lead in bees and honey (Tong *et al.*, 1975), however, the source of the lead was aerial deposition rather than contaminated soil.

Unlike lead, PCBs can bioaccumulate in organisms and biomagnify through food chains (Risebrough *et al.*, 1968; Peakall, 1975; Eisler, 1986). Adult June beetles collected using a light trap near Area 9 Landfill had relatively high levels of PCBs. Bioaccumulation of soil contaminants have been reported in larval June beetles or grubs (Heida *et al.*, 1985), but monitoring contaminant levels in June beetle adults collected using light traps has not been previously reported. An advantage of monitoring adult beetles is that direct contact of personnel with contaminated soil is not required. This is important since proper access to Superfund sites requires special training and experience. The major disadvantage to monitoring adult beetles caught in light traps is that bioaccumulation factors can not be calculated directly. For example, June beetle adults may fly some distance between daytime refuges and nighttime mating and feeding areas although these flights are generally kept at a minimum (Forbes, 1907). Nonetheless, an increased influx of adult beetles from areas remote to the contaminated site would "dilute" the level of contamination in pooled samples. Monitoring soil contaminants using adult June beetles provides an excellent index of soil contamination, but is unlikely to be used as a means of quantitative assessment of soil contaminant levels.

June beetles are important components of terrestrial ecosystems. Moles and other soil predators forage extensively on larval June beetle grubs (Tashiro, 1987). Massive emergence of adult May and June beetles also provides a food source in terrestrial communities, especially for nocturnal

avian and mammalian predators. Phillips (1966) found that beetles accounted for 100% of the big brown bat (*Eptesicus fuscus*) with June beetles making up 80% of the total. Keeler and Studier (1992) found that June beetles could provide soul source nutrition for pregnant brown bats, which could exacerbate problems of chemical contamination. No studies have investigated links between chemical contamination in adult June beetles and toxic effects in bats.

PCB profiles in adult beetles closely resembled the PCB mixture in soils from Area 9 Landfill. Higher organisms can often metabolize PCBs thus changing congener profiles (Hansen, 1987; Tanabe *et al.*, 1987). The lack of change in the PCB profiles between soil and beetles could be indicative of a lower capacity of these organisms to metabolically alter these compounds. Other insects have shown a low specific activity of this enzyme complex, relative to mammals (Benke and Wilkinson, 1971; Gilbert and Wilkinson, 1974).

Neither brood nor forager honey bees bioaccumulated PCBs in this investigation. This was unusual considering the high degree of volatilization of PCBs from soil (Lewis *et al.*, 1985), adsorption of PCBs on plants (Buckley, 1987), and other investigations of PCB bioaccumulation in honey bees. Anderson and Wojtas (1986) found PCB residues up to 56 ppm in dead bees collected from several apiaries. They also detected PCBs in brood comb and honey. The absence of PCB bioaccumulation in honeybees may be attributed to several factors. First, aerial transport may not be an important form of exposure at the site. However, this is unlikely given the high degree of exposure observed for caged crickets on Area 9 Landfill (McKee, 1992). Second, honeybees may have foraged away from the contaminated site thus avoiding exposure. Bees forage in a variable mosaic that can include an area 2 km in radius from the hive (Bromenshenk, 1992). Although, this may contribute to the absence of residues in this study, honeybees were noted foraging in large numbers on goldenrod growing directly on the Area 9

Landfill. At present we have no adequate explanation for the absence of PCB residues in honey bees at Area 9 Landfill.

The previous discussion regarding PCBs and invertebrates consider their importance in food chain transfer of contaminants. Relatively little information is available on toxicity of PCBs to invertebrates. Laboratory bioassays are important tools for establishing levels of contaminants necessary to produce toxic effects in the field. Whole body concentrations of persistent contaminants from laboratory studies can provide a useful benchmark for assessing hazard *in situ*. Paine *et al.* (1993) found significant mortality occurred in house crickets exposed to 1000 ppm Aroclor 1254 in the soil and that this mortality was associated with whole body concentrations of about 150 ppm wet weight. Moriarty (1969) reported significant mortality in the grasshopper, *Chorthippus brunneus*, exposed topically to 200 µg of PCBs. Whole body concentrations associated with this exposure concentration ranged from 100-300 ppm. These data combined with results from our study suggest that a benchmark concentration for mortality in terrestrial insects is in the range of 100-300 ppm. Residue studies by Rhett *et al.* (1988) showed that the LC50 was associated with whole body concentrations of 930 ppm PCB in earthworm tissue (wet weight), which suggests that earthworms may be more tolerant to PCBs than insects.

Application of benchmark levels in risk assessments require field measurement of contaminant concentration. Watson *et al.* (1985) investigated terrestrial fauna in an area containing PCBs in soil up to 6300 ppm. Shield-backed bugs (Homoptera: Scutelleridae) and ants (Hymenoptera: Formicidae) had the highest concentrations of PCBs ranging from 13.9 to 60.0 ppm wet weight. Crickets were found to have whole body concentrations of 0.31 ppm PCBs. We report whole body concentrations for June beetle adults ranging up to 22 ppm. Based on the laboratory benchmark toxicity levels, we expect the likelihood of

acute mortality to be low for the terrestrial insects investigated in these studies.

The absence of severe effects on epigeic invertebrates was supported by an invertebrate survey performed at Area 9 Landfill and reported elsewhere (McKee, 1992). No significant decrease in diversity or richness of insects at Area 9 Landfill compared to a reference site (Olin). Similarly, no significant decrease was observed in the number of field crickets, *Gryllus pennsylvanicus*, trapped on the Area 9 Landfill compared to the reference site (Paine *et al.*, 1993).

Although acute hazard was neither predicted or observed for epigeic invertebrates, organisms residing in the soil are at risk based on results of the earthworm bioassay. Our data with earthworms indicate that 37% mortality can be expected at a soil concentration of 300 mg of Aroclor 1254/kg in soil. This toxicity level is similar to the 14 day earthworm LC50 of 240 ppm in soil reported by Rhett *et al.* (1987). These data indicate that mortality of earthworms is likely in areas with soil contamination exceeding about 100 ppm.

White-footed mouse

Exposure of white-footed mice to PCBs in the environment is well documented. Batty *et al.* (1990) reported PCB body burdens ranging from 0.4 to 4.2 ppm, and Watson *et al.* (1985) found levels ranging from nondetectable to 3.0 ppm. Greichus and Dohman (1980) trapped mice near two transformer salvage companies and reported liver PCB levels ranging from 9.5 to 17.2 ppm and muscle levels ranging from 3.8 to 6.9 ppm. McKee (1992) found PCB levels as high as 22 ppm in adult white-footed mice trapped from Area 9 Landfill at Crab Orchard NWR. Residue levels from this study, demonstrate not only that high levels of exposure can occur in mice trapped on contaminated sites, but also that mice collected some distance from a site can have significant residues.

O'Brien and Gere (1988) estimated that small mammals living on contaminated soil at Job Corp Landfill would have daily intake of about 46.1 PCBs mg/kg. Estimates for particular routes of uptake were 1.10 mg/kg for inhalation, 43.3 mg/kg for ingestion, and 1.7 for dermal exposure. Uptake at Area 9 Landfill was estimated to be 39.1 mg/kg/day. Simmons and McKee (1992) measured carcass concentrations of white-footed mice exposed to dietary Aroclor 1254 in the laboratory. Table 20 shows the relationship between dietary intake and carcass concentrations. At the lowest concentration (2.5 ppm) the carcass concentration was about 5X the dietary intake. At the higher test concentrations the carcass concentrations were about 0.5X the daily ingestion rates. Since O'Brien and Gere's estimated uptake rates, are similar to the uptake rate observed at the higher test concentrations by Simmons and McKee (1992), we could expect some animals to have carcass concentrations of around 20 mg/kg (0.5 X 39.1). Indeed we observed animals with carcass concentrations in this range, providing evidence that O'Brien and Gere's daily uptake rates are reliable.

Table 20. Relationship between carcass concentration and daily ingestion rates of PCBs for white-footed mice following 21 days of exposure to Aroclor 1254 in the diet[a].

Diet Concentration mg PCB/kg diet	Carcass Concentration mg PCB/kg weight	Daily Ingestion mg PCB/kg weight/day
2.5	2.0	0.4
25	18.6	40
50	39.1	80
100	73.2	160

[a] Data from Simmons and McKee (1992). Daily ingestion rate based on an average of 0.16 g food/g body weight (Coburn and Treichler, 1946).

O'Brien and Gere (1988) estimated that inhalation would be about 1.1 mg/kg/day comprising 3.5% of the total daily uptake by small mammals at Area 9 Landfill. According to their model, dermal uptake was minimal and ingestion was the principal mode of uptake. The predicted concentration in air over an uncontrolled landfill containing 1 µg PCBs/g soil was estimated to be 11 $\mu g/m^3$ (USEPA, 1986) assuming an average wind of 10 mile per hour. The arithmetic mean and geometric means for the soil samples analyzed in this study at Area 9 Landfill (Figure 1) are 2320 and 71.8 mg/kg, respectively. Assuming a linear relationship between soil concentration and air concentration, the air over the landfill would contain 25,520 $\mu g/m^3$ PCBs for the arithmetic mean and 790 $\mu g/m^3$ based on the geometric mean. Lewis *et al.* (1985) reported air concentrations ranging from 577 to 1053 $\mu g/m^3$ in the air near the ground (2 cm above). Cubbage (Personal communication) has measured air concentrations near the ground of around 350 $\mu g/m^3$ at Area 9 Landfill. These data suggest that white-footed mice at Area 9 Landfill are exposed to air concentrations of PCBs around 350 $\mu g/m^3$. USEPA (1986) estimates a breathing rate of 0.10 m^3/day for a 30 g mouse. Based on these numbers, the daily ingestion of PCBs by mice would be 1.2 mg/kg/day, comparing very well with O'Brien and Gere's estimation of 1.1 mg/kg/day.

Relatively few studies have investigated the biological consequences of PCB exposure in wild-caught white-footed mice. Batty *et al.* (1990) showed hepatomegaly in some mice collected from a PCB contaminated site. Moreover, the authors reported for adult males a positive correlation of 0.81 between PCB body burden and relative liver weight. This correlation coefficient is similar to that observed for adult males in this study. Although a correlation coefficient for females was not reported by Batty *et al.* (1990), data from this study suggests that the correlation coefficients will be even higher for females than for males.

EROD activity in white-footed mice tended to level off with increasing

body burdens of PCBs yielding a "plateau effect" (Figure 7). Lubet *et al.* (1991) observed the plateau response in female F344/NCr rats following dietary exposure to Aroclor 1254. Simmons and McKee (1992) observed a plateau response for EROD activity in *P. leucopus* following dietary Aroclor 1254 exposure. The cause of the plateau response is not known, but may reflect inhibitory action of certain PCB congeners. Inhibition of enzyme induction has been demonstrated through the antagonistic action of certain PCB congeners at the Ah receptor (Bannister *et al.*, 1987; Davis and Safe, 1989). Similar plateau responses were not observed for PROD activity.

The hepatic effects data provides empirical evidence that PCBs are affecting white-footed mice *in situ*. The case for establishing PCBs as the etiological agent can be strengthened using laboratory investigations, especially those that link body burdens to biological effects. Several laboratory investigations have been reported in which white-footed mice were exposed to Aroclor 1254. Sanders and Kirkpatrick (1975) reported decreased pentobarbital sleep time in white-footed mice exposed 21 days to the lowest dietary concentration of Aroclor 1254 tested, which was 100 ppm. Body burdens were not measured and a no observed effect concentration (NOEC) for pentobarbital sleep time was not identified. More recently, Simmons and McKee (1992) reported carcass concentrations associated with hepatic effects in white-footed mice exposed to Aroclor 1254 in the diet. The NOEC for hepatomegaly following 21 days of dietary exposure was 2.1 ppm Aroclor 1254 in white-footed mice carcasses. The 2.1 ppm body burden was associated with exposure to 2.5 ppm Aroclor 1254 in the diet. Hepatic EROD activity was the most sensitive parameter measured with the NOEC below 2.1 ppm Aroclor 1254 in carcasses.

Based on body burden information generated in the study by Simmons and McKee (1992), EROD induction would be expected in wild caught white-footed mice with carcass concentrations of 2.1 ppm PCBs and higher. Hepatomegaly

would be expected at higher body burdens, probably around 10 ppm PCBs. These suppositions are supported by hepatic effects observed in white-footed mice collected at Area 9 Landfill and the Job Corp Landfill. Batty *et al.* (1990) reported increased liver weights in animals from a contaminated area, however, a lower range of whole body PCB concentrations (2 to 4 ppm) were associated with hepatomegaly than predicted based on the laboratory investigation by Simmons and McKee (1992). This discrepancy may be explained based on differences in field exposure (i.e., route and duration of exposure). Collectively, these studies strengthen the "weight of evidence" that liver effects observed in wild caught mice is caused by exposure to PCBs.

Hepatomegaly and monooxygenase enzyme induction are adaptive responses that can aid an organism in coping with environmental adversity. Therefore, establishing that these responses are caused by PCB exposure does not necessarily imply that survival, growth, or reproduction of white-footed mice will be reduced. One way to link PCB induced changes in liver to survival, growth, and reproduction is through the use of chronic laboratory investigations. Chronic studies with Aroclor 1254 have shown reproductive impairment in white-footed mice exposed to a dietary concentration of 10 ppm (Linzey, 1987; 1988). Although the author mentioned that liver enlargement was observed in some individuals, the effect was not quantified. Body burdens were not measured in the bioassays, so that direct comparisons to field collected animals is not possible. However, based on the shorter laboratory study by Simmons and McKee (1992), it is likely that carcass concentrations would be in the range of 1 to 10 ppm PCBs. Since field body burdens have been observed in this range, it is likely that some reproductive effects are occurring. Several *in situ* studies have implicated reproductive effects. Batty *et al.* (1990) reported impairment of reproduction for white-footed mice at a Michigan hazardous waste site as evidenced by low juvenile to adult ratio in the population and a significant decrease in testes weight. Other investigators have reported reproductive failure in wild mammals as a result

of PCB exposure (Gilbertson, 1988).

These data suggest that biological effects in the form of hepatic alterations and possible reproductive depression in white-footed mice is likely at relevant environmental concentrations of PCBs. Based on laboratory and field investigations, the NOEC for EROD activity (<2.1 mg Aroclor 1254/kg wet weight) and reproduction of white-footed mice are probably very similar. Therefore, EROD activity provides a sensitive biomarker for investigating the extent of PCB exposure in field populations and also may be reflective of adverse biological effects.

Lead was not measured in white-footed mice in this investigation because data reported elsewhere (McKee, 1992) showed that white-footed mice collected at Area 9 Landfill, Job Corp Landfill, the Old Refuge site, and Fire Station Landfill did not have carcass levels in excess of 1.61 µg/g dry weight. Several authors have used whole carcasses of white-footed mice to monitor lead contamination (Beyer *et al.* 1985; Clark, 1979). In these studies, whole body concentrations of lead in excess of 5 µg/g dry weight were associated with contaminated areas. Although whole body residues of lead can be used to detect lead contamination, more sensitivity may be achieved by using tissue specific concentrations (Kisseberth *et al.*, 1984; Scanlon, 1987; Welch and Dick, 1975). Based on these data, lead does not appear to accumulate to a large extent in white-footed mice occupying areas with lead contaminated soils.

Starlings and other birds

The starling, an abundant exotic passerine, has been suggested as a sentinel model for other avian species in agroecosystems (Robinson *et al.*, 1988) and at hazardous waste sites (Kendall, 1989). Stickel *et al.* (1984) investigated lethality as a function of Aroclor 1254 in tissues of starlings,

cowbirds (*Molothrus ater*), red-winged blackbird (*Agelaius phoeniceus*), and the common grackle (*Quiscalus quiscula*). Starlings were found to be the most sensitive species investigated, however, whole body concentrations associated with mortality were considerably higher than observed in this study. Mortality of adult birds was associated with whole body concentrations of 172 to 1,120 ppm compared to body concentrations ranging up to 3 ppm in nestlings from the present study. Based on these observations, it is unlikely that PCB exposure will result in acute mortality of starlings at the hazardous waste sites on Crab Orchard NWR.

Laboratory investigations with standard avian species have not shown PCBs to be particularly potent reproductive toxicants. Dietary NOECs were 50 ppm for Japanese quail and northern bobwhites (Eisler, 1986) and 25 ppm for mallards (Custer and Heinz, 1980). Despite the relative insensitivity of reproduction in these species, several field studies in the Great Lakes Basin have indicated that PCBs, at least certain congeners, can cause reproductive effects in the field. The proposed mechanism by which reproduction is affected is through embryotoxicity and parental behavior. Two case studies will serve to illustrate these mechanisms.

Reproductive failure was noted in a Lake Ontario Herring Gull (*Larus argentatus*) colony in 1966 (reviewed in Gilbertson, 1988; 1989). Field observations verified the presence of embryonic mortality, congenital deformities, and loss of eggs. Many of the symptoms resembled the chick edema disease that had been identified in poultry exposed to dioxins and PCBs. These symptoms include reduced weight gain, droopiness, hydropericardium, subcutaneous edema, peritoneal edema, and swollen liver (Gilbertson *et al.*, 1991).

Reproductive failure of a Forster's tern colony near Green Bay, WI was suspected since about 1973. Kubiak *et al.* (1989) reported that reduced

hatching and fledging success was correlated with organochlorine contaminants. The authors distinguished experimentally between intrinsic and extrinsic effects on egg development. Intrinsic effects refer to direct toxicant effect on the developing embryo, whereas extrinsic effects principally refers to the attentiveness of parental birds. Reproductive failure of the tern colony was a result of both intrinsic effects, with symptoms similar to chick edema disease, and extrinsic effects principally related to intensity of incubation.

Starlings in the present investigation exhibited reproductive failure. No evidence of developmental anomalies were noted. Embryos that survived to hatch, were not deformed and were likely to survive until fledging. Eggs that did not hatch were examined to determine the stage of development. All embryos stopped developing at an early stage (i.e., 3 to 5 days). Since those individuals that hatched were not abnormal, and those that did not hatch, stopped developing at a relatively early stage, we suspect that extrinsic factors are the principal cause of the reduced hatching success. Abandonment was observed for several nests early in the incubation period and starling adults at other nests showed erratic incubation habits. At Area 9 Landfill, where effects on starling reproduction were noted, the odor of PCBs is strong. It is not known to what extent the changes in incubation behavior is related to toxic effects of internalized contaminants or avoidance of the pungent odor.

Ecological risk assessment

Ecological risk assessment (ERA) can be defined as a "process that evaluates the likelihood that adverse effects may occur or are occurring as a result of one or more stressors" (USEPA, 1992). Regarding the CERCLA process, as amended by the Superfund Amendments and Reauthorization Act of 1986 (SARA), several statutory mandates require that ERAs be conducted (USEPA, 1989b). The major statutory provisions are listed below:

1. The statutes require that remedial actions selected for a site be sufficient to protect human health *and the environment*,

2. Statutes require compliance with applicable and appropriate requirements (ARAR's) which entails consideration of numerous Federal and State laws and regulations concerning natural resource preservation and protection when evaluating possible response actions, and

3. SARA calls upon the EPA to notify Federal natural resource trustees of negotiations with potentially responsible parties and to encourage trustees' participation in the negotiations if a release or threatened release may result in damages to protected natural resources.

A distinction should be made between ecological risk assessments (ERA) and ecological risk management (ERM). The ERA process is concerned with providing information on the type and magnitude of ecological damage in a particular situation. The ERM can be defined as "the process of decisionmaking that attempts to minimize ecological risks without undue harm to other societal values" (Suter, 1993). Although these are separate processes, it is prudent to select ecological endpoints for the ERA that can be relatively easily incorporated into risk management decisions. Endpoint selection becomes even more important when Natural Resource Damage Assessments

(NRDA) (#3 above) are to be conducted. The types of endpoints that should be measured for ERAs to support NRDAs are outlined in Part 11, Section 11.62 in the Federal Register (Federal Register, 1986). Those types of endpoints used to support injury claims for biological resources (section f) can be divided into three categories:

1. the hazardous substance caused the biological resource or its offspring to have undergone at least one of the following adverse changes in viability: death, disease, behavioral abnormalities, cancer, genetic mutations, physiological malfunctions (including malfunctions of reproduction), or physical deformations; or

2. the hazardous substance exceeds action or tolerance levels established under section 402 of the Food, Drug, and Cosmetic Act, in edible portions of tissue; or

3. the hazardous substance exceeds levels for which an appropriate State health agency has issued directives to limit or ban consumption of such organisms.

The first category pertains specifically to assessment of ecological effects and is relevant for the data presented in this report. Several criteria have been established (Federal Register, 1986, p. 27736) to ascertain the strength or legality of injury claims relative to #1 above and are presented in abbreviated form below:

- The biological response must be a commonly documented response resulting from exposure to the hazardous substance.
- Exposure to the hazardous substance is known to cause this biological response in free-ranging organisms.
- Exposure to the hazardous substance is known to cause this biological

response in controlled experiments.

- The biological response measurement is practical to perform and produces scientifically valid results.
- The injury must be established by statistical significance between the biological response in samples collected at a contaminated site versus a control site.

The biological responses reported in this investigation that support injury determination as specified in the Federal Register are shown in Table 20. The pathway by which these environmental receptors are exposed to PCBs will likely vary according to the species. All routes of uptake, inhalation, ingestion, and dermal, are suspected of contributing to toxicity and body burdens.

Table 21. Category of injury and supportive evidence establishing endangerment for wildlife at Crab Orchard NWR.

Category of Injury	Supporting Evidence
Death- 11.62,F,4,i,E- Established in accordance with section on **laboratory toxicity testing**.	Supported by Earthworm bioassay using soil collected from control and Area 9 Landfill.
Physiological malfunction- 11.62,F,4,v,B- Established in accordance with section on **reduced avian reproduction**.	Supported by several endpoints in starling investigation including fledging success and hatching success at Area 9 Landfill.
Physical deformation- 11.62,F,4,vi,C- Established in accordance with section on **internal whole organ and soft tissue malformation**.	Supported by significantly enlarged livers in white-footed mice collected at Are 9 Landfill.

CONCLUSIONS

PCBs, but not lead, readily move from contaminated soils into the terrestrial community. Pre-remediation levels were established in this report for June beetle adults, tree bark, honeysuckle, white-footed mice, starlings and Canada geese. June beetles and tree bark are particularly good monitoring organisms because they are relatively easy to collect and will probably be only moderately impacted by remediation activities.

PCBs at the landfills were associated with biological effects in earthworms, white-footed mice, and starlings. Acute risk was apparent for soil dwelling invertebrates. Hepatic effects were evident in white-footed mice occupying Area 9 Landfill and Job Corp Landfill. Carcass concentrations of PCBs found in white-footed mice were also likely to be associated with decreased reproduction, based on laboratory studies. Reproductive effects, in the form of reduced hatching success, were documented in starlings occupying nest boxes next to Area 9 Landfill. The reduced hatch appeared to be related to decreased parental attentativeness rather than direct effects on embryos.

The ecological risk assessment clearly establishes risk to individual organisms associated with landfills at Crab Orchard NWR, especially those containing PCBs. Individual risk is high for organisms occurring on or near the landfills. The risk of population decline over a large geographic area is lower than individual risk, since the landfills comprise a relatively small portion of the surface area. Consequently, localized effects will likely be compensated for when considering larger spatial scales.

More important than localized effects of the landfill chemicals on individual organisms is the export of PCBs from the landfills. USEPA (1986) estimated an emission rate of 1.13×10^{-10} g/cm^2/second for an uncontrolled landfill with 1 µg/g PCBs in the soil. The Area 9 Landfill is about 1

hectare, so the estimated release rate for the landfill is 980 g/day. This is probably a conservative estimate since the soil contamination is considerably higher than 1 µg/g. Since inhalation uptake is an important route of exposure, this source should be reduced as quickly as possible. This not only poses a risk to local wildlife, but also contaminates organisms located remote to these landfills. If incineration of contaminated soil will be years away, an interim (or final) remedy would be a cap to prevent the PCBs from so readily entering the terrestrial food chain.

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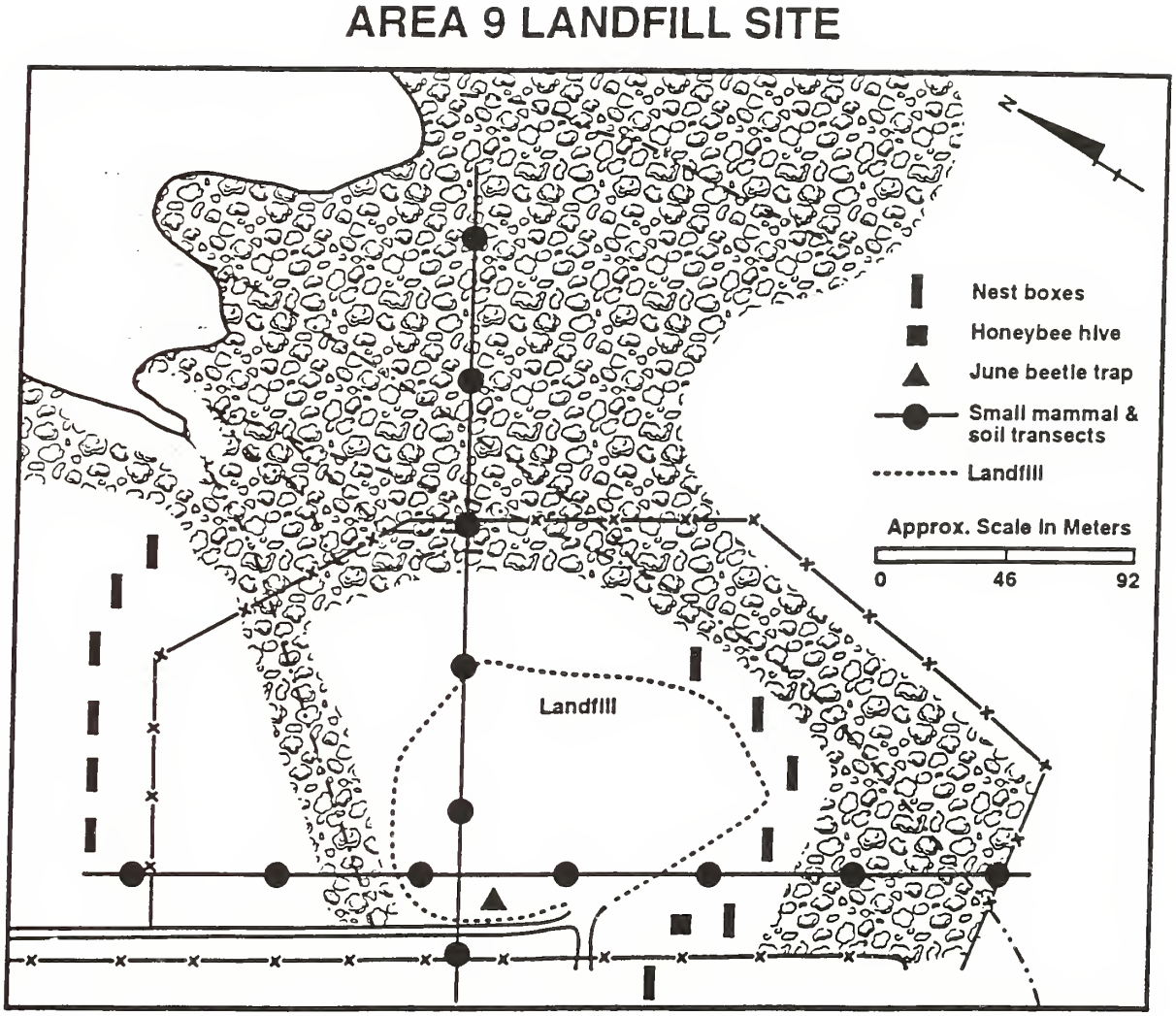
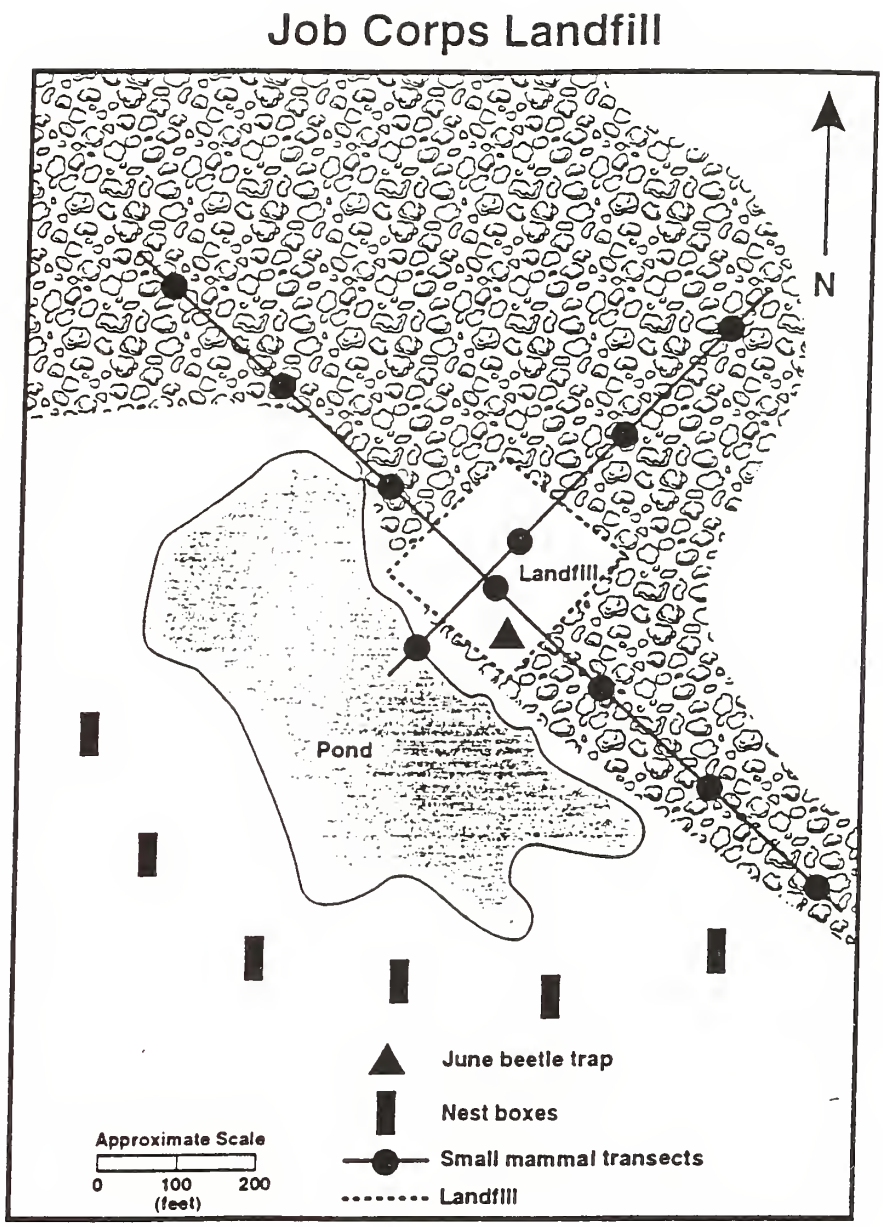
APPENDIX I

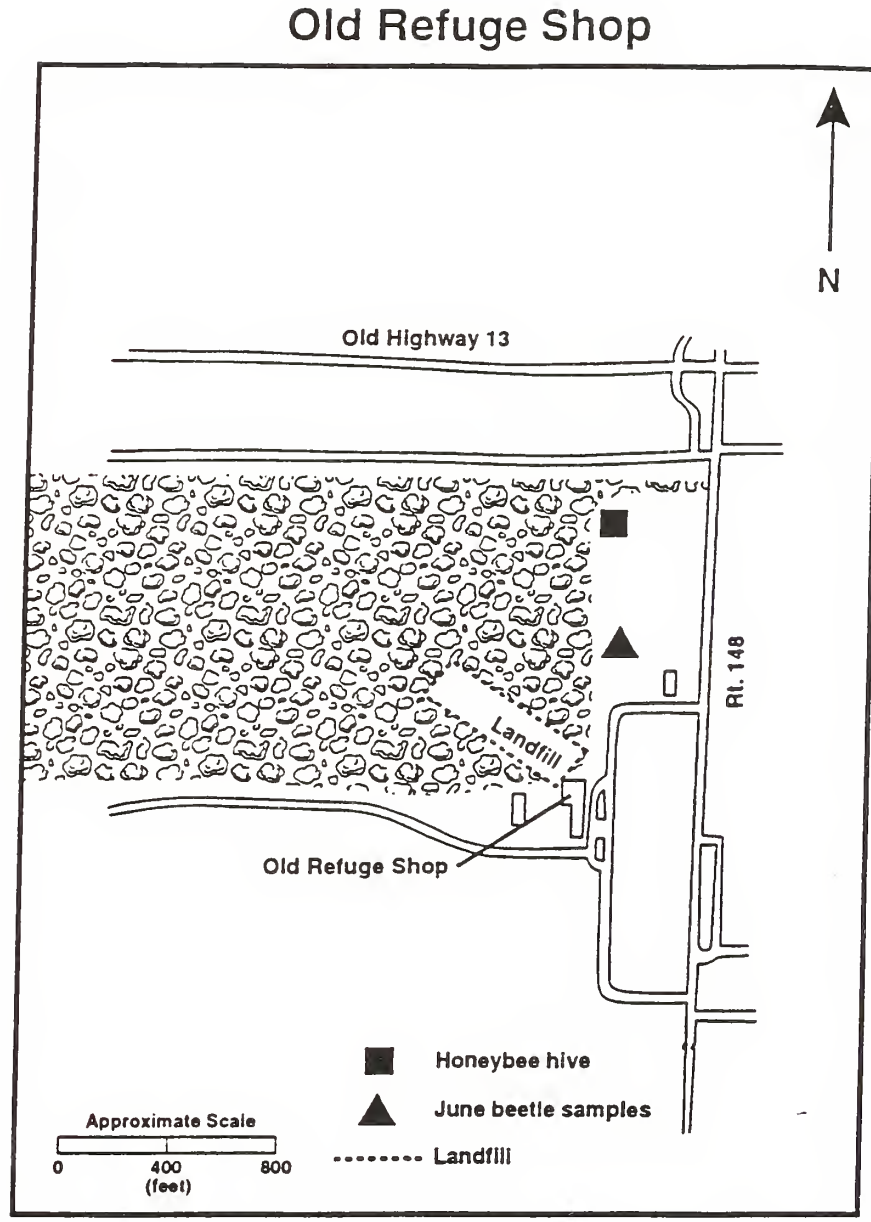
Site maps for study areas



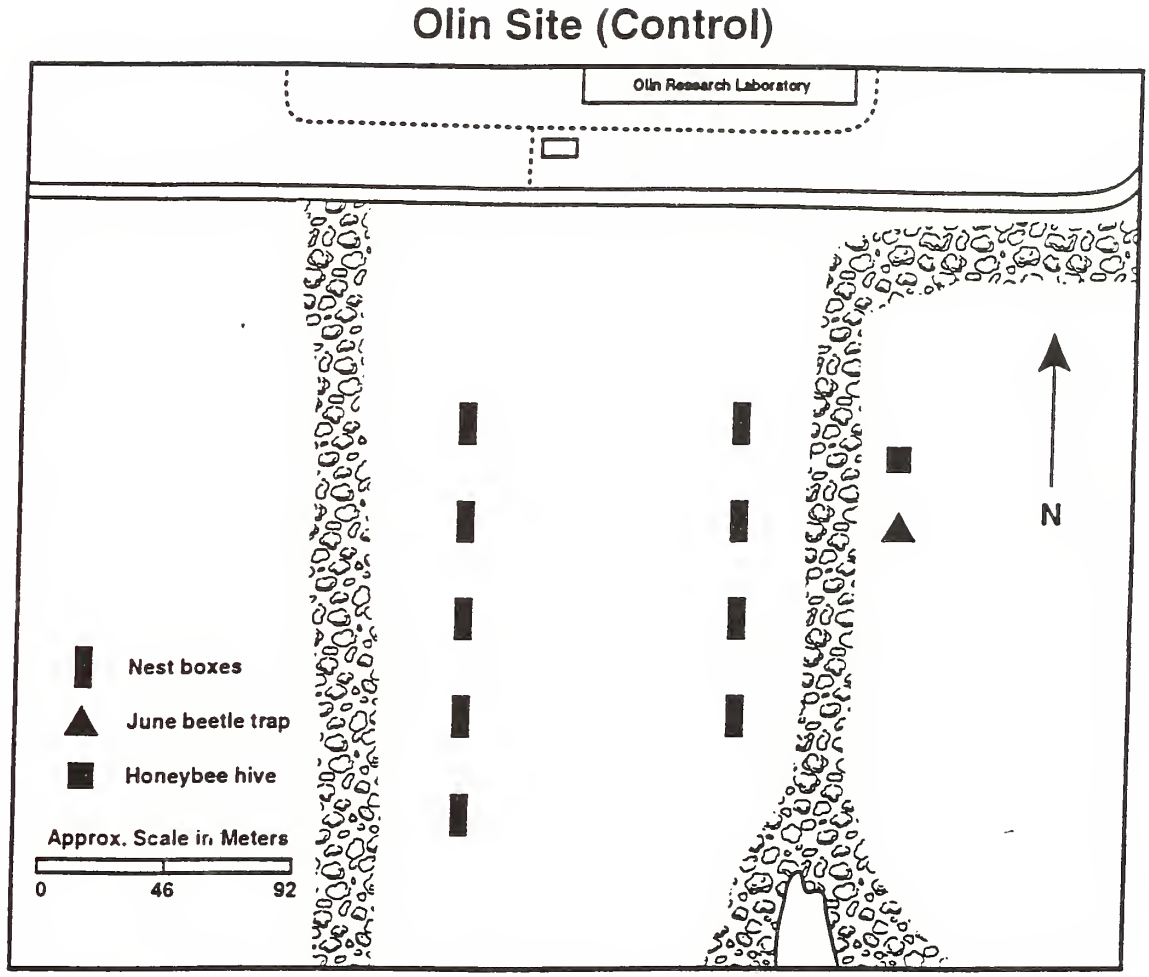
Map 1. Location of sampling areas for Area 9 Landfill at Crab Orchard NWR.



Map 2. Location of sampling areas for Job Corp Landfill at Crab Orchard NWR.



Map 3. Location of sampling areas for the Old Refuge Shop at Crab Orchard NWR.



Map 4. Location of sampling areas for the Olin reference site at Crab Orchard NWR.

APPENDIX II

Analytical reports and raw data

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